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EXPERIENCE WITH THE HORMONE TEST FOR PREGNANCY*

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There seems little if any need to emphasize the utility of a relatively reliable laboratory test for pregnancy as a diagnostic aid. Aside from furnishing a procedure to diagnose normal pregnancy at a much earlier date than is possible from the usual clinical signs, the hormone test for pregnancy serves particularly in those instances where early interference with pregnancy is indicated, as in some cases of diabetes, tuberculosis, renal disease, or cardiac lesion. Even more important perhaps is its service in the differential diagnosis of more or less acute abdominal conditions in which normal pregnancy, ectopic gestation, toxemia of pregnancy, hydatid mole or chorio-epithelioma, in other words conditions which yield a positive reaction, are possible factors. Finally it also serves as a test for the completeness of removal of the products of gestation, hydatid mole, or malignant growths originating from chorionic villi.

Current medical literature contains the records of a rapidly increasing number of statistical reports on the use of the test in the diagnosis of pregnancy as well as of case histories showing complex conditions which were clarified by the knowledge from the test of the presence or absence of a tubal or other extra-uterine conception.

As in the case of any other clinical laboratory test, its value is in direct ratio to its reliability and the proportion of errors, incorrect positives being more annoying and in some instances more hazardous than incorrect negatives.

The result of a number of tabulations by various authors shows

*Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6–9, 1932.

a striking similarity in the conclusion that the test under consideration is correctly positive in as high as 98 per cent of normal pregnancy cases. On the other hand there is no similarity in the experience with incorrect positives. A number of reports by others as well as our own results fortunately show no false positives. Other workers report a small number at most and account for them in one way or another, leaving relatively few in which the positive result could not be explained. In ectopic gestation the results in the different reports are not so similar as would be desirable, some showing as low as 50 per cent positive while others report a higher percentage of positive results. While it is generally stated that the death of the fetus is promptly followed by negative reactions, it is also reported by Wilson and Corner[3] and others that positive tests were obtained in cases where the dead fetus was retained for a considerable period.

No definite decision seems to have been reached as yet as to the shortest duration of pregnancy recognizable by a positive reaction but there are many reports quoting positive tests two or three days after the first day of the first missed menstruation, though this of course does not establish the date of conception. In few instances where pregnancy could be referred to a single date, a positive reaction has been reported in from ten to fourteen days after the coitus. Jones and Mugrage[2] report one case in which a positive reaction was obtained ten days after coitus. Wilson and Corner call attention to their experience of some negative reactions during the first month of pregnancy where positive reactions were obtained in from four to seven days later. It has generally been found that the reaction is most constant and pronounced during the early part of pregnancy and perhaps less so during the later months, persisting however until one or more days after delivery. Wilson and Corner claim that with the Friedman modification the positive reaction persists for twenty-four to seventy-two hours postpartum while with the Aschheim-Zondek test this period is from seven to eight days.

For the original Aschheim-Zondek test four or five immature white mice serve the purpose while for the Friedman modification one mature female rabbit is used. The advocates of the Fried-

man modification emphasize the difficulty attending the securing of a large number of mice of proper age at all times compared with the ease of having on hand a number of grown female rabbits properly segregated. Also that the Friedman test is complete in forty-eight hours while the Aschheim-Zondek technic requires 100 hours. The advocates of the Aschheim-Zondek method claim it to be a bit more sensitive and somewhat less liable to error in reading the result.

The first urine passed in the morning is the most desirable specimen for the test, taken at a time when the patient is not under medication. Catheterization is not necessary but a clean specimen in a clean container is essential. Many drugs taken by patients have an undesirable effect on the animal used for the test and some are promptly fatal to it. Several methods have been devised for the preliminary removal of objectionable qualities and others for concentrating the hormone in the urine to lessen the amount of specimen necessary to inject into the animal and to produce a more pronounced and possibly earlier result.

While the direct object of this communication is to detail our experience with the hormone test for pregnancy, it seemed desirable to present the above preamble as a premise. It is not proposed at this time to enter into a discussion of the advantage of one procedure over another or even to advocate refinements in the test. It must be apparent to all in this work that niceties of technic and improvements in the details of the test must result in still greater accuracy and possibly in the saving of time, though such modifications still require the test of further experience.

The following report concerns 1048 hormone tests for pregnancy in cases of various kinds in all of which a positive clinical diagnosis at least in so far as pregnancy is concerned was originally or subsequently established. We are indebted to Drs. F. C. Holden, F. W. Rice and H. C. Williamson of the obstetrical and gynecological services of Bellevue Hospital for a large part of the material. In 487 cases the original Aschheim-Zondek test and the Friedman modification were both done with the same specimen.

The report includes the technic used, a complete table of all tests done and the comparative results with the two tests in cases

of normal and abnormal pregnancy and allied conditions, of uterine and tubal disease without pregnancy and in beginning menopause, all cases offering differential diagnostic problems; also in acromegaly on account of the possibly associated endocrine activity. Our experience with the test during the first half of pregnancy is detailed. There is also a consideration of the mortality of the animals during the test period leading to the loss of the test, and finally there are conclusions prompted by our study.

In table 1 there is a list of all the tests we have made with the Aschheim-Zondek or the Friedman method of procedure. The

TABLE 1

COMPLETE LIST OF HORMONE TESTS FOR PREGNANCY

DIAGNOSIS	NUMBER OF TESTS	ASCHHEIM-ZONDEK OR FRIEDMAN TEST		CORRECT
		Positive	Negative	
				per cent
Normal pregnancy	764	716	48	93.7
Ectopic gestation	80	68	12	85
Hydatid mole	6	6	0	100
Beginning menopause	63	0	63	100
Fibromyoma uteri	33	0	33	100
Salpingitis	62	0	62	100
Amenorrhea unknown cause (no pregnancy)	24	0	24	100
Acromegaly	16	0	16	100

number of instances of a negative result in pregnancy cases was about 6.3 per cent which figure is due largely to conservatism particularly in our early work with the test and which probably also explains the fact that an incorrect positive was not found in a single specimen examined. It was this result however that prompted doing the test by both methods on the same specimen and this produced a materially higher degree of accuracy with again no incorrect positive reactions as will be shown later.

An analysis of the comparative results according to the duration of pregnancy shows the following in our series:

	PER CENT POSITIVE	
	Aschheim-Zondek	Friedman
First month or less	91	94
Second month	98	93
Third month	95	90
Fourth month	87	94
Fifth month	99	94

The earliest pregnancy in this series in which a positive result was obtained was one in which conception could not have occurred longer than 14 days prior to the test and the next earliest where the test was made two days after the first day of the expected menstruation. The technic used in most of the work with the Aschheim-Zondek test was that as originally recommended, namely four or five immature white mice weighing 8 to 12 grams each were injected with respectively 0.2, 0.25, 0.3, 0.3, 0.4 cc. of urine twice a day for three days and killed at the end of 100 hours after the first injection. In a number of instances more recently we have injected the mice with the same or slightly smaller amounts three times the first day, twice the second day, and once the third day, according to a published recommendation. Two were killed seventy-two hours after the first injection. If negative, in no instance as yet were the remaining mice positive at the end of the usual 100 hours after the first injection. In the Friedman modification, 10 cc. of urine were injected into the marginal ear vein of a female rabbit about three to four months old and the animal autopsied forty-eight hours later.

The specimen used for the test was the first morning urine with previous instructions that no drugs should be taken by the patient during the preceding twenty-four hours. Clean specimens in clean bottles were generally obtained and aseptic collection was not demanded. Alkaline specimens were acidified and all were placed in an ice-box for several hours and then filtered before use. Specimens from a distance had one drop of cresol added to each 50 cc. of urine. The method recommended by Zondek for the removal of toxic substances by shaking the specimen with ether with or without the subsequent addition of glucose was not used in

the series now reported, nor was any attempt at concentration of the hormone made as also suggested by Zondek[4] and by Eberson.[1] These improved methods appear trustworthy and interesting and will doubtless have to do with the ultimate refinements in the procedure.

The animal mortality during the test period is of interest and though probably avoidable with the recent refinements in technic, is still embarrassing when it occurs. In our series of cases twelve sets of mice and ten rabbits died during the test period, a mortality of 3.59 per cent and 3.17 per cent respectively. In the series when the test was done concurrently with both methods,

TABLE 2

COMPARATIVE ANALYSIS OF 487 COMPLETED TESTS WITH BOTH THE ASCHHEIM-ZONDEK AND FRIEDMAN METHODS

DIAGNOSIS	TOTAL	ASCHHEIM-ZONDEK TEST			FRIEDMAN MODIFICATION			BOTH TESTS		
		Positive	Negative	Correct	Positive	Negative	Correct	Either positive	Both negative	Correct
				per cent			*per cent*			*per cent*
Normal pregnancy	365	343	22	94	339	26	92.9	358	7	98.1
Ectopic gestation	38	33	5	86.8	31	7	81.6	35	3	92.1
Hydatid mole	3	3	0	100	3	0	100	3	0	100
Tests in nonpregnant cases (see table 1)	81	0	81	100	0	81	100	0	81	100

the mice and rabbit both died in only six instances, a mortality of 1.1 per cent which is negligible. In practically all cases these animal deaths were referable to decomposition or bacterial growth in the specimen, presence of acetone and diacetic acid, or to the use of drugs by the patient such as ergot, quinine, arsenic, luminol, morphine, codeine or aspirin.

In the interest of greater accuracy as previously stated, we undertook a series of 487 cases in which the Aschheim-Zondek and the Friedman modification were used concurrently, with results as detailed in table 2. These figures demonstrate the advantage of using both methods by the appreciably higher per-

centage of correct positive reactions in normal pregnancy and particularly in cases of ectopic gestation, a complete absence of incorrect positive reactions and a minimal loss of animals during the test period. Thus the combined methods show 98.1 per cent positive reactions in normal pregnancy compared with 94 per cent Aschheim-Zondek alone and with 92.9 per cent Friedman modification alone. In ectopic gestation there was a similar improvement, 92.1 per cent with the combined method compared with 86.8 per cent Aschheim-Zondek alone and with 81.6 per cent Friedman modification alone.

CONCLUSIONS

It was demonstrated in a relatively large series of cases that incorrect positive results can be avoided. The concurrent use of the Aschheim-Zondek test and the Friedman modification results in a higher proportion of correct positive reactions and a lessened liability of loss due to the death of animals during the test period. For these reasons the combined test is advocated until additional refinements make it unnecessary, particularly in cases presenting difficulties in differential diagnosis in which surgical intervention may be indicated.

REFERENCES

(1) Eberson, F.: A rapid method for diagnosis of early pregnancy from urine. Proc. Soc. Exp. Biol. & Med., **28:** 407–409. 1931.

(2) Jones, R. H., and Mugrage, E. R.: The Aschheim-Zondek pregnancy test. Am. Jour. Clin. Path., **1:** 379–384. 1931.

(3) Wilson, K. M., and Corner, G. W.: The results of the rabbit ovulation test in the diagnosis of pregnancy. Am. Jour. Obst. & Gyn., **22:** 513–519. 1931.

(4) Zondek, Bernhard: Zur Methodik der Schwangerschafts-Reaktion aus dem harn durch Nachweis des Hypophysenvorderlappenhormons. 1. Fällungschnellreaktion—2. Entgiftung des Harns. Klin. Wochenschr., **9:** 964–966. 1930.

THE RESULTS OF TWO YEARS EXPERIENCE WITH THE FRIEDMAN TEST*

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Two years ago we reported before this society a series of fifty clinical cases in which the Friedman modification of the Aschheim-Zondek test for pregnancy had been used with gratifying results.[9] This series was extended to 150 cases in a subsequent paper,[10] and last year had reached a total of 390 tests, of which 278 had been checked by the subsequent clinical course.[11] The present report is a summary of our experience in approximately 1000 tests, of which 865 have been checked by subsequent clinical course. This marked numerical increase in the utilization of the test is further demonstrated in that our first series represented six months' work with repeated requests to clinicians for coöperation. During the past year we have received without solicitation over 600 specimens for diagnosis, and splendid coöperation in the reports of the subsequent clinical course.

This experience has been duplicated by numerous individuals who have carefully investigated the test, and may also be illustrated in the tremendous increase in the number of papers reporting experiences with the test.

In the preparation of our first report of cases, Friedman's fundamental work[5] demonstrating the scientific principles of the test was the only available reference to this method, although we have since ascertained that Jares[7] had confirmed Friedman's work. The reports of experience with the test since then have become quite numerous, and the value of the test has been demonstrated in thousands of cases.

Furthermore the evolution of titles under which the Friedman

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test has been reported, while numerous and varied, were logically standardized in the report of Magath and Randall.[8] The active principle in the urine of pregnant women which will induce a characteristic change in the ovaries of immature female white mice, and its absence in practically all other conditions, represents the fundamental fact of the test, and was demonstrated, amplified, standardized, and popularized by Aschheim and Zondek. Friedman demonstrated that the ovaries of the rabbit might likewise be used as a biological indicator of the presence of this principle if the urine was injected intravenously. This represented a decided advance in the technique of the test. Therefore all tests in which the rabbit is the animal used as the biological indicator originate from the work of Friedman.

Another factor which has been responsible for the variety of names under which the Friedman modification has been reported, is the number of investigators who began working on the test as soon as Friedman's original paper concerning the availability of the rabbit appeared. It is pertinent that in Friedman's first papers no standardized technique or details of the actual test were proposed. Therefore each investigator was confronted with the necessity of working out his own technique. Schneider[12] was impressed with the maturing factor of the active principles in the urine of pregnant women as demonstrated originally by Aschheim and Zondek and therefore began using immature female rabbits. Friedman,[5] having suggested the substitution of the rabbit for the mouse, was apparently influenced by the original technique employed with mice and therefore made repeated injections and examined the ovaries of the rabbits at autopsy. This technique is undoubtedly best for technicians who are not familiar with animal experimentation. Wilson and Corner,[13] with long experience in the physiology of reproduction, examined the ovaries by laparotomy sixteen hours after injection with excellent results.

Our experience has passed through various stages prompted by expediency. Our present technique more nearly approaches that of Wilson and Corner than any other.

METHOD

We have always preferred adult rabbits weighing four pounds or more. However, occasionally there are younger rabbits in the lots we have received, and our results with these are as good as with those obtained from larger rabbits. In our earlier injections we used 5 cc. of urine intravenously and operated on the rabbit under anaesthesia eighteen to twenty-four hours later. If examination of the ovaries revealed negative results we reinjected 5 cc. of urine and reoperated after an additional twenty-four hours. In an occasional case we discovered a delayed reaction, but with more careful original examination of the ovaries we believe these could have been diagnosed. In the hope of avoiding a delayed reaction and obtaining a more decisive end result, we now inject 10 cc. of urine and routinely wait forty-eight hours; this eliminates approximately 50 per cent of our operative procedure, which is necessitated by the large increase in the number of specimens submitted for examination. Furthermore, since we routinely utilize all negative rabbits for subsequent tests until a positive reaction is obtained, we save our rabbits from undue trauma. Rabbits presenting a negative test may be immediately reinjected; if the urine is positive, they will react as well as animals which have had no injection. This means a saving of more than 50 per cent in animals. They may be used in this manner until they appear sick or develop troublesome infection or adhesions. Usually this method does not permit more than three successive tests on one rabbit. Occasionally when short of animals we reinject positive reacting rabbits, if seven days have elapsed following the previous operation. These cases are then subsequently controlled by injection of the same urine into a fresh rabbit when the new lot arrives. While we have encountered no errors by this method, we do not advocate its usage except in emergency. In these cases, previous to the injection, we open the abdomen and note the number, size, and distribution of hemorrhagic follicles from the previous test; forty-eight hours after this injection the ovaries are carefully compared with their previous condition. Positive cases are easily diagnosed; negative cases require careful consideration.

Examination of the ovaries of the rabbit by operation rather than autopsy has proved highly satisfactory. The animal is strapped to the board, anaesthetized with ether, the foot of the board is elevated so the intestines and oviducts drop toward the diaphragm, the hair is clipped short in the lower portion of the abdomen, and an antiseptic solution applied in this area. The abdominal incision is about two inches long, the oviducts are picked up with forceps and pulled down until the ovaries are visible. After examination the wound is closed with silk in two layers, an antiseptic reapplied to the wound, and the rabbits are placed in their cage without any dressing. The entire procedure is carried out by one man, rarely requires more than fifteen minutes, and the animals universally do well. We have outlined these details in the evolution of our technique rather minutely, not because they are better than that of others, but to emphasize that apparent variations in technique are not material, but have been largely developed by experience or as a result of local conditions.

A more material variation in the test is reported by Brown,[3] in which the blood serum of pregnant women is injected intravenously into female rabbits with examination by autopsy twenty-four to thirty-six hours later. We used blood serum in the place of urine in 1930 with a small series of cases, and in those cases found it as reliable as the intravenous injection of urine. We considered it more cumbersome than the Friedman modification, and clinicians expressed such a great preference for the urine test that we discarded the serum test.

Another modification of the original Aschheim-Zondek test is that of Davis and Ferrill[4] in which immature female rats from twenty-five to thirty days of age are used rather than immature female mice. In our original paper[9] we noted our experience with "immature white rats was similar to that obtained with white mice," and so far as we were concerned, this technique gave no improvement as a practical clinical test over the original Aschheim-Zondek test. The accuracy of this modification, however, has been further demonstrated by Dr. E. P. Durrant[14] of the Department of Physiology of Ohio State University. This method may be of value in some communities where the rabbit and mouse are not so available as the white rat. The local animal supply is certainly a factor in the determination of what modification of the original test may be used, and if the biological principle remains unchanged, the results should not differ materially.

The complete series of tests outlined in table 1 represent the work of our laboratory and the laboratory of White Cross Hospital of Columbus, Ohio.

In any test where the check of accuracy or control depends upon the interpretation of a number of different individuals, the opportunity for error is undoubtedly increased. If, in addition, we appreciate the subsequent varied course which may present itself to the clinician for interpretation, correlation, and judgment as to the accuracy of the diagnosis submitted from this test, the possibility of error must likewise increase. For example, a married woman went to a clinician complaining of two missed periods and desiring to know if she was pregnant. Although she had been married seven years, examination revealed an intact hymen.

Further examination revealed the early usual presumptive signs of pregnancy. A specimen of urine submitted for examination gave a positive test. Later the woman went to another physician for puerperal care. The uterus was enlarged to about a three or four months pregnancy, but as time went on the size did not increase. Finally about the seventh month of her supposed pregnancy a second specimen of her urine was received for examination which proved to be negative. The physician concluded the first test had been in error and that the patient had a tumor

TABLE 1

SUMMARY OF RESULTS

White Cross Hospital	392		1,002
Department of Pathology, Ohio State University	610		
Subsequent clinical history accurately reported	854	865	1,002
Subsequent clinical history equivocal	11		
Subsequent clinical history absent	137		
Tests checked by subsequent clinical course			
Test reported positive	423		865
Test reported negative	442		
False positive tests	5 or 1.20% error		
False negative tests	15 or 3.40% error		
Equivocal tests	11		
Maximum error of series	31 or 3.59%		
Minimum error of series	20 or 2.32%		
Hydatid moles	3		

in her pelvis. The patient refused any operative investigation; near the end of the eighth month of her supposed pregnancy she went into labor and spontaneously delivered macerated twins estimated to have been of about three months development. A lack of the complete history would have branded one of the tests as incorrect. Fortunately such cases are rare. In eleven of the cases cited in the table subsequent clinical history was such that the clinicians were unwilling to state whether or not the test had been correct. Of these six were positive and five were negative. They were followed either by atypical menstruation or abortions.

Less careful physicians in some of the other cases must have encountered the same thing and interpreted it according to the report which they had received. In 148 cases the subsequent clinical history is not known.

We encountered five false positive reactions and three hydatid moles,[1] which gave positive reactions. Of the false positive reactions one was diagnosed menopause, one an ectopic pregnancy which did not exist as proved by operation, the uterus as well as the tubes being normal, one as gall bladder disease, and two presumably functional amenorrhea.

The false negative reactions have constantly been more frequent in our experience than the false positive reactions. In fact in the first 130 positive reactions which we obtained there was not one in error. Bland, First, and Roeder[2] obtained a correct negative in 93.2 per cent and a correct positive in 82.4 per cent with the original Aschheim-Zondek test. With the Friedman modification we have obtained a correct negative in from 95.5 per cent to 96.6 per cent, and a correct positive in from 97.4 per cent to 98.8 per cent according to whether or not we include the eleven questionable cases as in error. We have insisted upon no report on the test being made positive unless it is unquestionably positive. This probably accounts for the larger number of false negatives in our series. We feel that these results are significant in that they represent a transition from a biological problem to a standard clinical test without undue variation in accuracy.

SUMMARY

1. The rapid and widespread usage of the Friedman test is briefly outlined.

2. No significant variation in Friedman's technique has appeared.

3. The transition from the stage of biological trial to a standard clinical test has been accomplished without significant variation in accuracy.

REFERENCES

(1) ASCHHEIM, SELMAR: The early diagnosis of pregnancy, chorion-epithelioma and hydatidiform mole by the Aschheim-Zondek test. Am. Jour. Obst. and Gynec., **19:** 335–342. 1930.

(2) BLAND, P. B., FIRST, A. AND ROEDER P.: A comparative study of the Aschheim-Zondek and Mazer-Hoffman tests for early pregnancy. Am. Jour. Obst. and Gynec, **23:** 83–89. 1932.

(3) BROWN, T. K.: A proposed modification of the Aschheim-Zondek "pregnancy test". Am. Jour. Obst. and Gynec., **23:** 379–385. 1932.

(4) DAVIS, M. E. AND FERRILL, H. W.: Some observations on the Aschheim-Zondek test in the diagnosis of pregnancy. Am. Jour. Obst. and Gynec., **23:** 567–574. 1932.

(5) FRIEDMAN, M. H.: Mechanism of ovulation in the rabbit. Am. Jour. Physiol., **90:** 617–622. 1929.

(6) FRIEDMAN, M. H. AND LAPHAM, M. E.: A simple rapid procedure for the laboratory diagnosis of early pregnancies. Am. Jour. Obst. and Gynec., **21:** 405–410. 1931.

(7) JARES, J. J.: Studies on ovulation induced by extracts of the hypophysis and by the urine of pregnant women. Anat. Rec., **45:** 264. 1930.

(8) MAGATH, T. B. AND RANDALL, L. M.: Friedman's hormone test for pregnancy. Jour. Am. Med. Assn., **96:** 1933–1935. 1931.

(9) REINHART, H. L. AND SCOTT, E.: The hormone test for pregnancy. Am. Jour. Clin. Path., **1:** 113–126. 1931.

(10) REINHART, H. L. AND SCOTT, E.: A modification of the Aschheim-Zondek test for pregnancy. Jour. Am. Med. Assn., **96:** 1565–1567. 1931.

(11) REINHART, H. L. AND LYONS, W. F.: Scientific exhibit American Medical Association, Philadelphia Session.

(12) SCHNEIDER, P. F.: A hormone test for the diagnosis of early pregnancy. Surg., Gynec. and Obs., **52:** 56–60. 1931.

(13) WILSON, K. M. AND CORNER, G. W.: The results of the rabbit ovulation test in the diagnosis of pregnancy. Am. Jour. Obst. and Gynec., **22:** 513–519. 1931.

(14) Personal communication.

THE COMPARATIVE VALUE OF MONOCHLORBENZENE AND THYMOL WHEN USED WITH FLUORIDE AS PRESERVATIVES OF BLOOD FOR CHEMICAL ANALYSIS.

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The sugar of the blood undergoes such a marked reduction in concentration beginning soon after the blood is drawn that delayed analyses yield worthless results. Moreover, the ammonia content increases with the standing of blood and, since total non-protein nitrogen and urea are determined as ammonia, erroneous figures for these constituents are obtained when analyses are not made on fresh blood. For these reasons, the desirability of finding methods for rendering these blood chemical constituents more stable, so that the analyses may be delayed to a convenient time, has been the incentive for considerable investigation in recent years. Many of the methods suggested have proven more or less satisfactory, but there is a lack of confirmation of the efficacy of any single preservative for all of the non-protein nitrogenous constituents and sugar. The literature on the subject is extensive and a brief review will serve to show which methods have thus far proved most successful.

Among the first preservatives suggested was formaldehyde, which was used by Denis and Aldrich[4] to prevent glycolysis. However, Bock[2] reported that only a very few brands of commercial formaldehyde were suitable for this purpose, the majority causing an appreciable reduction of the Folin-Wu sugar reagent. Splatt[24] likewise found formaldehyde to be unsatisfactory for preventing a change in the sugar concentration, as there was a continual increase in sugar or other reducing substances in the blood up to seven days, at which time coagulation of the specimen occurred.

Picric acid was suggested by John[10] as being successful in the dual capacity of protein precipitant and preservative for sugar. Obviously, this substance could not be used when sugar was to be determined by other methods than those employing picric acid.

Potassium fluoride used alone was found satisfactory for the preservation of sugar and carbon dioxide combining power for seven days by Major[16] (5) when the specimen was collected and stored in a sterile manner. Denis and Beven[5] reported that sodium fluoride, when added to oxalated blood, acted as an efficient preservative of sugar for ninety-six hours and of other constituents for forty-eight hours. Roe, Irish, and Boyd[19] also studied the preservative action of fluoride and found that 10 mgm. of sodium fluoride per cubic centimeter of blood prevented changes in non-protein nitrogen, uric acid, creatinine, sugar and cholesterol in sterile blood for at least ten days, but that satisfactory preservation was not obtained even with 20 mgm. of sodium fluoride per cubic centimeter of blood under non-sterile conditions.

Sander[22] proposed sodium fluoride and thymol in amounts of 0.02 gram of the former and 0.002 gram of the latter per cubic centimeter of blood and found it to be an efficient general preservative for a period of five to six days. The effectiveness of this method has been amply verified by other workers and has practically come to be the standard procedure. Denis and Hume[6] found it satisfactory in preventing a destruction of the sugar for five to six days, while Cameron and Williamson[3] reported it efficient for the nitrogenous constituents as well as for sugar for a period of three to four days. Nadler, Starr, and Tukey[17], using this combination of thymol and sodium fluoride, found it suitable for the preservation of sugar for six to fourteen days, and John[11] found it more satisfactory than other methods for the same purpose. Splatt, using this method to prevent glycolysis, found that the concentration of the sugar was maintained practically at a level up to fourteen days when the determinations were performed by the Maclean method. Schwentker[23] reported thymol-fluoride to be an efficient preservative for

sugar in one-half the amounts advised by Sander when the blood is analyzed according to the Folin-Wu procedure, but that the sugar values, when they are determined by the Lewis-Benedict[14, 15] method, are from 7.5 to 70 per cent too high. In this article, Schwentker also stated that he was able to confirm the results of Sander on the efficacy of thymol-fluoride in preventing changes in the concentration of the nitrogenous blood chemical constituents. Kilduffe and Springer[12] reported that thymol and sodium fluoride as used by Sander interfere with urea determinations by the Folin-Wu technic. Rose and Schattner[20] found that blood preserved with sodium fluoride and thymol lost 50 per cent of the sugar in three days and all of it in six days. Thus, the literature shows a lack of uniformity of opinion concerning the preservative qualities of thymol and sodium fluoride.

Rose and Schattner obtained excellent preservation of sugar with a mixture of monochlorbenzene and sodium fluoride. By this preservative combination, the blood sugar was kept within 0.015 per cent of its original value for fifteen to twenty days, and in two instances up to seventy-two days with no greater variation. However, this report contained no mention of the influence of these combinations of substances on the preservation of the nitrogenous blood chemical constituents.

In addition to the method just mentioned, Rose and Schattner[21] recently outlined a procedure particularly adapted for sugar preservation in samples for use in sugar tolerance tests. Blood samples of 0.1 to 0.2 cc. were stirred into a desiccated mixture of 15 grams of sodium sulfate and 200 mgm. of sodium fluoride contained in small vials. Samples thus dehydrated gave tests within 2 to 5 per cent of the initial analysis from a few days after collection up to three months.

The purpose of the present investigation was: (1) to find a method for the preservation of the nitrogenous blood chemical constituents as well as the sugar when the blood specimens are sent through the mails, and (2) to find some material which could be added to oxalated bloods to preserve effectively all of these constituents for at least two days when the blood is stored in an ice box.

EXPERIMENTAL

Human blood was used in all of our investigations, and the blood specimens were collected without regard to the time of day or to the length of time elapsed since the last food. No effort was made to employ sterile syringes or containers. By means of a Luer syringe, 10 to 20 cc. of blood were drawn from a cubital vein and transferred immediately to a 50 cc. Erlenmeyer flask, or to a wide mouthed bottle of the same capacity in which the anticoagulant and preservative agents had previously been placed. The anticoagulant was distributed in the bottles by adding the required volume of a solution of known strength of potassium fluoride and then evaporating off the water. The remaining compound of the preservative combination, the thymol or monochlorobenzene, was then placed in the containers just previous to the addition of the blood.

For no particular reason, we employed potassium fluoride in our studies instead of the more customarily used sodium fluoride. In checks which were made later to determine the comparative action of the two compounds, no difference in preservative ability was found. Neither was there any apparent difference in their respective action toward the analytical reagents. It was thought possible that the comparatively large amount of potassium in the form of potassium fluoride that was required to prevent clotting of the blood might interfere with the uric acid and creatinine determinations as suggested by Benedict,[1] but no such interference was ever discernable.

The analytical methods employed were for the most part those recommended by Folin and Wu[8,9] as modified for use with the Peebles-Lewis colorimeter.[15,18] The blood was precipitated according to the Folin-Wu procedure and analyses for sugar, total non-protein nitrogen, urea nitrogen, uric acid, and creatinine were performed on this filtrate. In the determination of uric acid, the reagent proposed by Folin and Trimble[7] was used. In the micro-Kjeldahl determination for total non-protein nitrogen, the Koch-McMeekin method[13] was employed. After sufficient blood had been reserved for an initial analysis, the stoppers of the containers in which the specimens were stored were sealed by dipping them in melted paraffin to prevent any possible evaporation of the liquid. At certain intervals the containers were unsealed, sufficient blood was withdrawn for analysis, and the containers were immediately resealed for further storage.

Several substances not heretofore suggested were investigated for possible powers of preservation. Chlorazene, hexylresorcinol, and furfural caused reduction of the copper-containing reagents to such a degree that they proved valueless. Menthol, while it had no deleterious effect on the reactions involved, failed to exhibit any preservative action.

While we were engaged in this attempt to find a new preserva-

tive agent, the report of Rose and Schattner[20] appeared with statements of the excellent results obtained by the use of monochlorbenzene and sodium fluoride in the preservation of sugar in the blood. A few tests in our laboratory with monochlorbenzene and potassium fluoride proved that not only was the sugar satisfactorily preserved, but that this combination prevented a change in other blood chemical constituents as well. Since monochlorbenzene gave evidence of such exceptional possibilities as a blood preservative material, we decided to abandon further search for a new substance and to give our attention to a comparative study of the preservative properties of monochlorbenzene and thymol on fluorided and oxalated bloods.

The first comparison consisted in determining the respective preservative properties of thymol-fluoride and monochlorbenzene-fluoride on blood stored at room temperature (25°C.). The results obtained showed no superiority of one preservative method over the other. Consequently, the detailed tabular report of 113 determinations on eight blood specimens was not deemed of sufficient worth to be included in this report. However, the results may be summarized as follows:

With thymol-fluoride the preservation of sugar was satisfactory for six to eight days, and in one case for twenty-one days. The maximum period of satisfactory preservation for the nitrogenous blood chemical constituents proved to be six to eight days. Monochlorbenzene-fluoride held the blood sugar content without appreciable change for seven to twenty-six days, thereby confirming the report of Rose and Schattner.[20] Non-protein nitrogen remained practically constant up to seven days, and the variation up to fourteen days was not large. The results for urea-nitrogen were nearly as satisfactory, and the preservation of uric acid and creatinine were excellent for even longer periods.

A second comparison consisted in an attempt to preserve blood while storing specimens in an incubator at a temperature of 37°C. Under these conditions there was a failure in preservation of the blood specimens with the use of either the thymol-fluoride or the monochlorbenzene-fluoride combination. During

these tests conducted at elevated temperatures, clots or coagulum appeared in the specimens preserved with monochlorbenzene-fluoride but were absent in specimens containing thymol-fluoride.

One other means of obtaining comparative results was tried, that of shipping the blood specimens through the mail and thereby making tests under conditions which the bloods would actually undergo, were they to be sent to the laboratory from surrounding localities. The specimens were collected and placed in 50 cc. bottles as in previous experiments. After sufficient blood was

TABLE 1

PRESERVATIVE EFFECT OF THYMOL-FLUORIDE ON BLOODS SHIPPED THROUGH THE MAILS*

(Thymol, 0.02 gram and potassium fluoride, 0.275 gram per 20 cc. of blood)

SPECIMEN NUMBER†	GLUCOSE		NON-PROTEIN NITROGEN		UREA NITROGEN	
	Initial analysis	Final analysis	Initial analysis	Final analysis	Initial analysis	Final analysis
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
61 (6 days)	95	70	32	39	15	17
63 (6 days)	88	67	42	47	18	21
40 (7 days)	98	90	31	38	14	19
42 (8 days)	92	78	30	40	14	15
56 (9 days)	90	88	26	34	12	17
59 (9 days)	89	72	26	37	12	18

* Figures for uric acid and creatinine were omitted from this table to conserve space. Practically no change occurred in these constituents.

† Figures in parentheses represent the number of days elapsing between initial and final analyses.

removed for an initial analysis, the containers were carefully packed in mailing tubes and sent to various destinations which afforded journeys of from four to twelve days, as indicated in tables 1 and 2. In most cases, identical bloods were used for testing both preservative combinations. This was accomplished by drawing 20 cc. of blood from each patient and discharging 10 cc. into each of two bottles containing the respective preservatives. After removing sufficient blood for immediate analysis, these pairs were mailed and upon their return a second analysis was performed.

The experimental results under actual mailing conditions are given in table 1 for thymol-fluoride and in table 2 for monochlorbenzene-fluoride. Comparing the figures of the two tables, it will be seen that with few exceptions the thymol-fluoride composite failed to preserve the blood chemical constituents effec-

TABLE 2

PRESERVATIVE EFFECT OF MONOCHLORBENZENE-FLUORIDE ON BLOODS SHIPPED THROUGH THE MAILS*

(Monochlorbenzene, 0.2 gram and potassium fluoride, 0.275 gram per 20 cc. of blood)

SPECIMEN NUMBER†	GLUCOSE		NON-PROTEIN NITROGEN		UREA NITROGEN	
	Initial analysis	Final analysis	Initial analysis	Final analysis	Initial analysis	Final analysis
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
72 (4 days)	85	81	42	46	16	17
73 (4 days)	86	85	48	49		
74 (4 days)	85	85	41	42	16	16
75 (4 days)	86	84	48	50	17	18
60 (6 days)	85	80	34	34	17	17
62 (6 days)	92	89	42	42	18	19
41 (7 days)	96	98	32	34	16	16
43 (8 days)	92	90	30	31	15	15
64 (8 days)	82	77‡				
65 (8 days)	88	80‡				
66 (8 days)	89	84‡	34	41‡		
68 (12 days)	98	96	34	36	14	17
69 (12 days)	98	95	34	38	14	17
70 (12 days)	90	92	33	35	14	16
71 (12 days)	89	91	33	39	14	16

* Figures for uric acid and creatinine were omitted from this table to conserve space. Practically no change occurred in these constituents.

† Figures in parentheses represent the number of days elapsing between initial and final analyses.

‡ Specimens contained a large amount of clotted material upon return.

tively even for six days, whereas, except in the cases noted below, the monochlorbenzene-fluoride accomplished excellent preservation for as long as twelve days. Attention should be called to the lack of preservation by monochlorbenzene-fluoride that occurred in specimens #64, #65, and #66. Each of these

bloods contained a large amount of clotted material that resembled the condition resulting when the blood specimens of a previous series of tests were kept in the incubator, and only a small amount of fluid blood was available for analysis. These specimens showed a decrease in sugar content and specimen # 66, the only one of the three allowing sufficient fluid for an analysis of the nitrogenous constituents, gave an increased total non-protein nitrogen. This occasional failure of the monochlorbenzene-fluoride to preserve blood would be more serious, were it not for the fact that evidence of the deterioration is shown by the physical condition of the specimen (occurrence of clots) even before analysis is performed. In contrast to this, the blood specimens in which thymol-fluoride was used gave no evidence of failure of preservation by their physical appearance.

The following explanation might be offered as to why under laboratory conditions so little difference was found, and yet under mailing conditions so decided a divergence occurred, in the comparative powers of preservation of thymol-fluoride and monochlorbenzene-fluoride. No temperatures between 25°C. and 37°C. were tried in preliminary work in the laboratory. Although there is no means of knowing the conditions to which blood specimens are subjected in the mail, it may well be that temperatures higher than 25°C. but lower than 37°C. are experienced and that the monochlorbenzene-fluoride acts as a preservative under such conditions whereas the thymol-fluoride fails. We see then that, even though the results obtained at room and incubator temperatures show no superiority of the one preservative over the other, the data obtained under mailing conditions prove conclusively that monochlorbenzene-fluoride is vastly superior to thymol-fluoride as a preservative.

Throughout this entire study 0.2 gram (0.18 cc.) of the monochlorbenzene was used to 20 cc. of blood. While this amount is not completely miscible with 20 cc. of water, no difficulty was encountered in the soiling of pipettes or containers even when a larger amount was used with the same volume of blood. It is probable that any excess in the blood is removed in the process of protein precipitation and that the amount remaining in the

filtrate is merely in the form of a saturated solution and thus has no effect on the glassware. The minimum effective amount of monochlorbenzene for the preservation of all constituents was found to be 0.1 gram per 20 cc. of blood but, since no interference was found with 0.2 gram, we have used this amount routinely in order to allow an excess for a safety factor.

TABLE 3

PRESERVATIVE ACTION OF MONOCHLORBENZENE-OXALATE ON BLOODS STORED AT A TEMPERATURE OF 0° TO 6°C.*

(Monochlorbenzene, 0.2 gram and potassium oxalate, 0.04 gram per 20 cc. of blood)

SPECIMEN NUMBER†	GLUCOSE		NON-PROTEIN NITROGEN		UREA NITROGEN	
	Initial analysis	Final analysis	Initial analysis	Final analysis	Initial analysis	Final analysis
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
26 (2 days)	86	88	28	30	15	17
30 (2 days)	70	74	29	30	12	13
32 (2 days)	102	97	27	26	13	12
34 (2 days)	98	97	28	27	13	13
37 (2 days)	105	103	30	28	13	13
38 (2 days)	94	92	31	32	13	14
18 (4 days)	93	87	27	28	17	17
19 (4 days)	100	104	31	33	12	13
26 (4 days)	86	88	28	29	15	16
32 (4 days)	102	103	27	28	13	13
34 (4 days)	98	98	28	27	13	13
37 (4 days)	105	106	30	29	13	13
38 (4 days)	94	91	31	30	13	14

* Figures for uric acid and creatinine were omitted from this table to conserve space. Practically no change occurred in these constituents.

† Figures in parentheses represent the number of days elapsing between initial and final analyses.

The other problem concerning which information was desired, as previously mentioned, was to find a preservative which when added to oxalated blood would, under conditions of refrigeration, prevent a change in the chemical constituents and thereby permit a postponement of analysis until a convenient time. Without such a preservative, it would be necessary to keep a technician

on duty in the blood chemistry laboratory on Sundays and holidays. On the other hand, if such a preservative material were known, it would be possible to take bloods on those days and store them in the ice box during the twenty-four to forty-eight hours until the next regular laboratory day, thereby making it unnecessary to conduct immediate analyses except when an emergency request was received. The relative efficacy of the two preservatives previously used, thymol and monochlorbenzene, were studied in this connection.

To our surprise, thymol when added to the oxalated bloods proved useless as a preservative. Even after so short a period of time as twenty-four hours none of the blood chemical constituents except uric acid and creatinine were maintained within satisfactory limits. The probable explanation of this failure is that at a temperature of 6°C. thymol is so slightly soluble in blood that no preservation could result. On the other hand, the addition to oxalated bloods of monochlorbenzene (0.2 gram per 20 cc. of blood) gave almost perfect preservation for as long as ninety-six hours. In table 3 it may be observed that without exception all constituents studied were preserved within narrow limits for this length of time. For longer periods than ninety-six hours preservation was not so perfect, but the shorter period was sufficient to fulfill the requirements desired.

SUMMARY

1. A combination of monochlorbenzene and potassium fluoride was found to be a more effective preservative for blood sugar, total non-protein nitrogen, and urea than thymol and potassium fluoride. Little change occurs in the uric acid and the creatinine with either preservative combination.

2. Blood specimens may be transported through the mails in journeys up to eight days with no appreciable change in either the blood sugar or the nitrogenous blood chemical constituents when a preservative combination composed of 0.275 gram of potassium fluoride and 0.2 gram of monochlorbenzene per 20 cc. of blood is employed. In journeys of twelve days, the sugar is well preserved, and only slight changes occur in the non-protein nitrogenous constituents.

3. Neither monochlorbenzene-potassium fluoride nor thymol-potassium fluoride will satisfactorily preserve blood specimens which are subjected to a temperature of 37°C. for twenty-four hours or longer.

4. If a blood specimen treated with monochlorbenzene-potassium fluoride has been subjected to high temperatures during passage in the mails, there will be a failure of preservation of the blood chemical constituents. Such blood will contain numerous small clots which will interfere with pipetting of the blood. The absence of such clots, therefore, offers a simple criterion as to successful preservation.

5. When preserved by the addition of 0.2 gram of monochlorbenzene to 20 cc. of blood, oxalated blood specimens may be stored in a refrigerator at 6°C. for ninety-six hours with practically no variation in the blood chemical constituents.

REFERENCES

(1) Benedict, S. R.: The determination of uric acid in blood. Jour. Biol. Chem., **51:** 187–207. 1922. (cf. footnote p. 194).

(2) Bock, J. C.: On the use of formaldehyde for the preservation of blood specimens. Jour. Biol. Chem., **59:** 73–76. 1924.

(3) Cameron, A. T., and Williamson, J. E.: The preservation of blood samples for chemical analysis. Canad. Med. Assn. Jour., **15:** 393–394. 1925.

(4) Denis, W., and Aldrich, M.: Note on the preservation of specimens of blood intended for blood sugar determinations. Jour. Biol. Chem., **44:** 203–206. 1920.

(5) Denis, W., and Beven, J. L.: Methods of preservation of specimens of blood intended for the determination of the nonprotein organic constituents. Jour. Lab. and Clin. Med., **9:** 674–679. 1924.

(6) Denis, W., and Hume, H. V.: On the nature of blood sugar. Jour. Biol. Chem., **60:** 603–612. 1924.

(7) Folin, O., and Trimble, H.: A system of blood anslysis: Improvements in the quality and method of preparing the uric acid reagent. Jour. Biol. Chem., **60:** 473–479. 1924.

(8) Folin, O., and Wu, H.: A system of blood analysis. Jour. Biol. Chem., **38:** 81–110. 1919.

(9) Folin, O., and Wu, H.: A system of blood analysis: A simplified and improved method for determination of sugar. Jour. Biol. Chem., **41:** 367–374. 1920.

(10) JOHN, H. J.: Methods of precision in the diagnosis of diabetes. Jour. Am. Med. Assn., **78:** 103–105. 1922.

(11) JOHN, H. J.: Preservation and transportation of blood for chemical study. Arch. Path. and Lab. Med., **1:** 227–236. 1926.

(12) KILDUFFE, R. A., AND SPRINGER, E. G.: Effect of blood preservatives on urea determination. Arch. Path. and Lab. Med., **4:** 396–397. 1927.

(13) KOCH, F. C., AND MCMEEKIN, T. L.: A new direct nesslerization of the Nessler-Folin reagent for ammonia. Jour. Am. Chem. Soc., **46:** 2066–2069. 1924.

(14) LEWIS, R. C., AND BENEDICT, S. R.: A method for the estimation of sugar in small quantities of blood. Jour. Biol. Chem., **20:** 61–72. 1915.

(15) LEWIS, R. C.: Two improved designs of Peebles-Lewis clinical colorimeter. Jour. Lab. and Clin. Med., **16:** 914–917. 1931.

(16) MAJOR, R. H.: Potassium fluorid as a preservative for blood. Jour. Am. Med. Assn., **81:** 1952. 1923.

(17) NADLER, W. H., STARR, P., AND TUKEY, G.: Glycemia as a guide in the treatment of diabetes mellitus. Arch. Int. Med., **36:** 579–584. 1925.

(18) PEEBLES, A. R., AND LEWIS, R. C.: A simple and accurate colorimeter for clinical use. Jour. Am. Med. Assn., **70:** 679–680. 1918.

(19) ROE, J. H., IRISH, O. J., AND BOYD, J. I.: The preservation of blood for chemical analysis by the use of sodium fluoride. Jour. Biol. Chem., **75:** 685–695. 1927.

(20) ROSE, A. R., AND SCHATTNER, F.: Preventing glycolysis in blood samples. Proc. Soc. Exp. Biol. and Med., **22:** 363–364. 1925.

(21) ROSE, A. R., AND SCHATTNER, F.: Preservative for small blood samples sent through the mails. Jour. Biol. Chem., **92:** xvii–xviii. 1931.

(22) SANDER, F. V.: The preservation of blood for chemical analysis. Jour. Biol. Chem., **58:** 1–15, 1923.

(23) SCHWENTKER, F. F.: Preservation of blood for chemical analysis. Arch. Path. and Lab. Med., **3:** 410–413. 1927.

(24) SPLATT, B.: A note on the preservation of samples of blood for sugar estimations. Med. Jour. of Australia, **59:** 73–76. 1924.

EVIDENCE IN SUPPORT OF THE NEURO-EPITHELIAL NATURE OF MOLES*

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Moles or nevi of the skin are easily accessible for macroscopic and microscopic study, yet one hundred years after the development of the achromatic microscope, observers still disagree as to their true biologic nature. From a study of their histology two main schools have arisen, one contending that they are of mesodermal origin and the other that they are of ectodermal origin. Among the chief supporters of the mesodermal origin are, Virchow,[31] Demiéville,[7] Ehrmann,[8] Ribbert,[25, 26] Recklinghausen,[24] Lubarsch,[17] Jadassohn[13] and Soldan.[28] Unna, in 1892 propounded the ectodermal theory; this was at first strongly opposed, but later drew to its support such able investigators as Krompecher,[15] Kromayer,[14] Dalla Favera,[5] Darier,[6] Marchand,[19] Gilchrist,[12] Bloch[3, 4] and Miescher.[23] In considering soft nevi or true moles Unna said: "All pigmented and nonpigmented, flat, just elevated nevi which are removed from infants and children, show a direct connection of the surface epithelium, and, in parts rich in follicles, of the prickle layer of the hair follicles, or even of the ducts of the coil glands, with the cellular cords of the nevus" (fig. 1).

Langerhans[16] described certain cells in the epidermis which bear his name. He found these cells to be pear-shaped, situated three to five layers above the basal layer in the rete malpighii and to have several processes, one directed toward the dermis similar to an axone, the others toward the stratum corneum similar to dendrites. He noted that they never contained pigment and manifested a special affinity for gold chloride, which led him to conclude that they were of a nervous nature. He was of the

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opinion that they were not epithelial cells, nor could he find any connection between the single axone-like process and the tactile corpuscles in the dermis.

Riehl, in 1884, discovered in the epidermis of man a dendritic cell; Bloch[3] described this cell in detail. Becker[2] also described the cell in a consideration of melanin pigmentation. The cell is situated in the basal layer and occasionally in the adjacent layers of the epidermis between the nondendritic cells. Its body, which

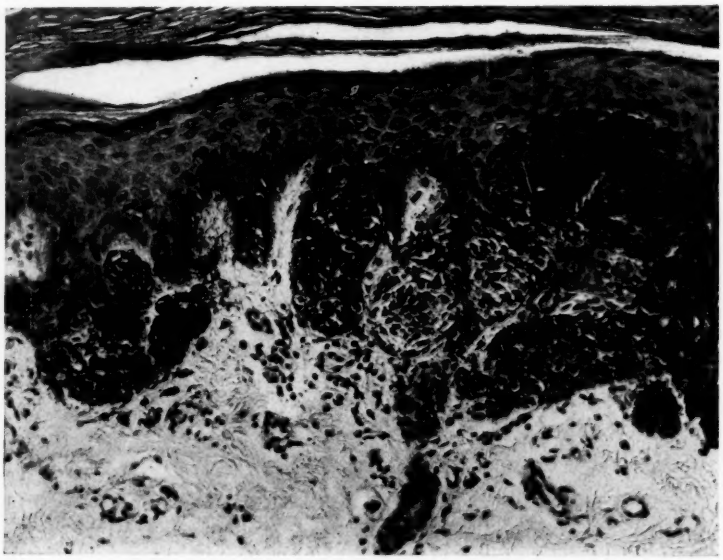

FIG. 1. PIGMENTED MOLE FROM THE TOE OF A YOUNG MAN
This shows the origin of the nevus cells from the stratum germinativum

is often irregular, contains varying amounts of melanin. The dendrites which vary from two to three or more also contain melanin, branch frequently, extend between the interstices of the cells and are said to communicate with one another. It is generally agreed that this cell is a true epithelial melanoblast, and unlike the Langerhans' cell is dopa positive and stains well with silver nitrate.

It was thought best to describe the Langerhans' and dendritic cells in the foregoing paragraphs since it is necessary to have a

clear conception of these two entities in order to understand the modification of the ectodermal theory of origin of moles which is to follow.

Simon[27] spoke of certain nevi as "nerve nevi" and thought that they were connected in some obscure way with the nervous system. This type of nevus was called by von Baerensprung,[1] "naevus unius laterus," and by Unna,[29, 30] "naevus linearis." These nevi were thought to have some relationship to trophic nerves. As a matter of fact, they have practically nothing in common with true moles and should not be considered in the same category. Soldan,[28] pointed out that pigmented spots of the skin are frequently the first signs of a fibromatous process of the connective tissue of the nerves of the skin, and that the pigmentation is probably a consequence of this fibromatosis. He also pointed out that neurofibromas develop as the result of proliferation of the connective tissue of the peripheral nerves and that from the fibromatous processes of the pigmented spots, warts, soft fibromas, pigmented nevi, and so forth, are developed. According to him the cordons and nests of nevus cells described by Unna and others, are neither epithelial tumors nor lymphangiofibromas of Recklinghausen, but neurofibromas.

Masson[19, 20] should be given full credit for bringing about a radical modification of the ectodermal theory of the origin of moles. It is his belief that they are formed essentially by the abnormal proliferation of the ends of the tactile nerves. He contends that they are not neurofibromas in the old sense that they arise by proliferation of the connective tissue intimately associated with nerves, as held by Soldan, but are composed of the syncytium of Schwann, the cells of which are partially neurotized. It is probable that the connective tissue of nerves referred to by Soldan is the same as that now spoken of as tissue of Schwann.

It seems pertinent here to call attention to an article written by Masson[20] on the Langerhans' cell in which he stated that the cell is capable of gold impregnation, which agreed with Langerhans' original description; however, he endowed it with properties which do not belong to it but do belong to the dendritic cell, namely, the ability to form melanin, as shown by Bloch's dopa reaction and

the staining of the melanin granules with silver nitrate. In other words, Masson created a hybrid cell out of the true Langerhans' and dendritic cells. The foregoing error, as previously pointed out by Bloch, has led to considerable confusion and no doubt has done much to discredit Masson's work on the nervous origin of moles.

It is Masson's belief that Langerhans' cells, or what he calls Langerhans' cells, the normal cells of Merkel-Ranvier and the epithelioid nevus cells are cells of the same nervous origin in which the function varies, and also that the cells of Langerhans as present in the normal epidermis are autonomous, or that they arise from, and give rise to Langerhans' cells and are not segregated and modified malpighian cells, as held by Unna, Kromayer and Darier. Masson further stated that a cellular nevus is able to grow by proliferation and differentiation of its own intradermal elements, without the epidermis participating. He observed a superabundance of neurites in nevi, and believed that their distribution among the nevus cells confirms the idea that the tumors correspond to a proliferation of nervous elements, and that since they present a form of differentiation comparable to tactile corpuscles, one can imagine that the nerves which ramify there are tactile. In his conclusions he stated that the epithelioid nevus cells are comparable to the cells of Merkel-Ranvier, the nevus corpuscles are comparable to the corpuscles of Wagner-Meissner, and that these two elements are associated in a syncytium with a system of plexiform and nonmyelinated nerve fibers attached to certain myelinated nerves of the dermis. It is his belief that all pigmented nevi are neuronevi and that all are neuromatous terminations of tactile nerves. He gave two distinct points of origin of nevi, one epidermal from his so-called Langerhans' cell, and the other intradermal or nervous. It is his belief that the elements arising from these respective points come together and fuse in the dermis.

We were prompted to write this paper by the microscopic evidence revealed in the examination of a soft papillary pigmented mole, 1.5 cm. in diameter that was situated in the skin in the postero-auricular region of a woman aged thirty-one years. Just

beneath the epidermis, this mole presented the usual alveolar masses of nevus cells with spheroidal hyperchromatic nuclei and scant cytoplasm. These cells which to us are in an undifferentiated state are called epithelioid cells by Masson and are thought by him to be comparable to the normal cells of Merkel-Ranvier

FIG. 2. PIGMENTED MOLE OF SKIN OF POSTERIOR AURICULAR REGION

Note the alveolar masses of undifferentiated cells, high in the dermis and considered by Masson to be comparable to the cells of Merkel-Ranvier. Directly continuous with these cells are structures which are described in figure 3.

(fig. 2). Lying deeper in the dermis but directly continuous with the undifferentiated nevus cells are cord-like structures which contain fibrillated bodies, the so-called lames foliacees of Masson (fig. 3). These bodies are made up of fine fibrillary processes which intermingle with each other. Intimately associated with

them are a few scattered flattened nuclei. It is reasonable to assume that these processes represent the cytoplasm or a differentiated product of nevus cells. Wherever these fibrillated bodies occur, the nuclei are sparse and small in contrast to the nuclei of the undifferentiated nevus cells which are usually collected into

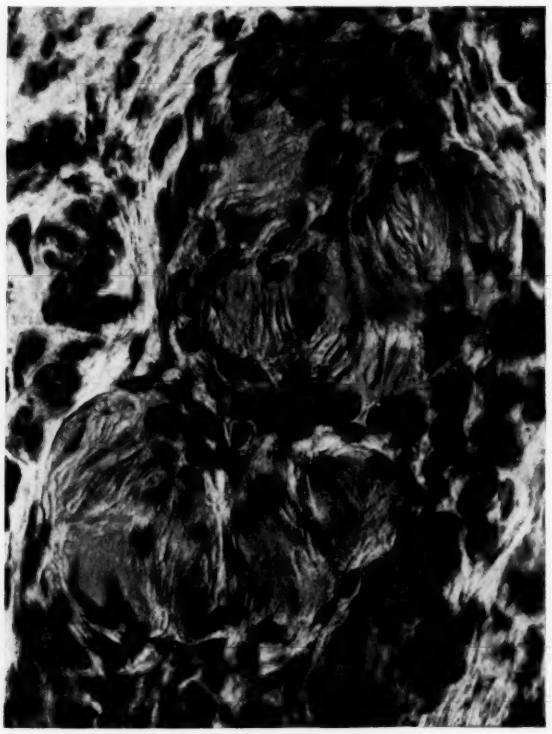

FIG. 3. HIGH POWER OF LAMES FOLIACEES, CONTAINING A TUFT-LIKE ARRANGEMENT OF FINE FIBRILS

alveolar masses and cordons, for the most part just beneath the epidermis, as pointed out by Masson who believes that these bodies are comparable to the Wagner-Meissner tactile corpuscles. Still deeper in the dermis but directly continuous with the cord-like structures containing the fibrillary bodies, are elongated bundles which Masson believes to be nonmyelinated nerves. Scattered throughout the nevus, but sparsely in the undifferen-

tiated cellular area is a very fine fibrillated structure which has the appearance of being the cytoplasm of nevus nuclei, with which it is in close contact. In the cord-like structures of another pigmented mole this tissue is very abundant (fig. 4). It is not connective tissue of mesoblastic origin but appears to be peculiar

FIG. 4. CORD-LIKE STRUCTURES IN A MOLE

Note the fine fibrillated tissue that resembles neuroglia, tissue of Schwann or the fine texture nervous tissue of a neurofibroma.

to the nervous system in that it resembles neuroglia, tissue of Schwann or the fine texture nervous element of a neurofibroma.

Various differential staining methods have been applied to the foregoing moles, with the idea of obtaining a specific reaction for nervous tissue. However, further proof of their nervous nature has not advanced beyond that already learned with the use of

hematoxylin and eosin. With the latter stains, the undifferentiated nevus cells present the same staining qualities as the regenerative protective epithelial cells. The fibrillated bodies and the fine texture fibrillary tissue, stain a deep orchid in sharp contrast to the pink of the true connective tissue fibers. With Van Gieson and the Castroviejo modification of the Cajal trichrome connective tissue stain, the nuclei of the undifferentiated nevus cells stain a Vandyke brown with the former stain and a purple with

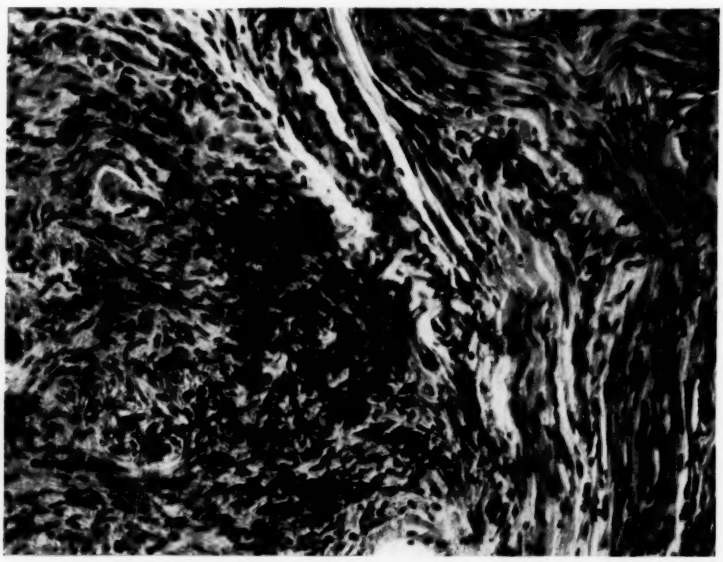

FIG. 5. PIGMENTED LESION OF THE SKIN OF THE DORSUM OF THE FOOT

This shows a picture typical of the blue nevus of Jadassohn in the left half and an intimately connected myelinated nerve in the right half.

the latter. The cytoplasm of these cells stains a pale yellowish green with both stains similar to that of the cytoplasm of regenerative protective epithelial cells. The fibrillary elements also stain a pale yellowish green with both stains in sharp contrast to the connective tissue which with Van Gieson stain is a light red, and with the modified Cajal stain there is a variation from pale blue to a bright green. These moles were stained with various silver and gold impregnation methods such as Orlandi, Cajal

and Bielschowsky, in an effort to bring out the nervous nature of the fibrillary elements. Mallory phosphotungstic acid stain and toluidin blue for neuroglia fibers were also used without avail. Recent investigations of Foot,[10,11] by silver impregnation methods have corroborated the findings of Masson.

In the recent examination of a pigmented lesion of the skin on the dorsum of the foot we noted areas typical of the blue nevus of Jadassohn, one area of which contained undoubted nerve tissue

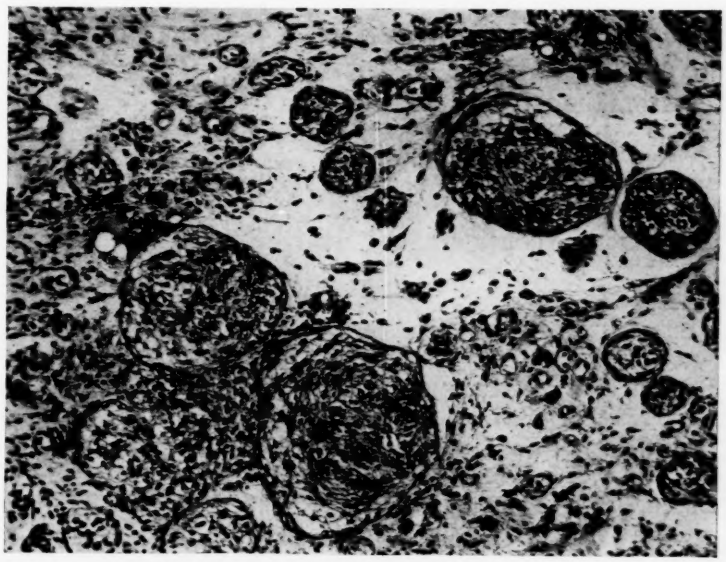

FIG. 6. ANOTHER AREA IN THE PIGMENTED LESION SHOWN IN FIGURE 5
Alveolar formations of typical nevus cells may be noted

that intermingled with the pigmented nevus elements (fig. 5). Other areas of this lesion disclosed alveolar formations of typical nevus cells (fig. 6).

In conclusion it may be stated that we agree with observers who hold that pigmented and nonpigmented moles or nevi and their malignant congeners the melanotic and amelanotic carcinomas or so-called melanomas are not of mesodermal origin nor are they of a connective tissue nature. We are also of the opinion that the so-called blue nevus of Jadassohn is probably not of mesodermal

origin but that it belongs to the same family as other pigmented nevi. It differs from the usual pigmented nevus in that its cells show a marked tendency to assume a spindle form thereby giving it a connective tissue effect. We agree with Unna and others who hold that moles are primarily of epidermal origin. We also agree with Masson to the extent that some moles show nervous elements; however, we are not in a position to agree with his original and ingenious hypothesis, that these nervous elements originate from the tactile nerves, neither can we agree that moles are of necessity neuronevi. It seems reasonable to assume that since the nervous system and the epidermis develop from the ectoderm, and since moles are of epidermal origin, that their spheroidal cells which we believe to be in an undifferentiated or embryonic state are atavistic to the extent that they are capable of differentiating into nervous tissue, and therefore in such light may be considered of a neuroepithelial nature.

REFERENCES

(1) Barensprung: Naevus unius lateris. Ann. d. char. Krankenh., **2:** 91–95. 1863.

(2) Becker, S. W.: Melanin pigmentation. Arch. Dermat. and Syph., **16:** 259–290. 1927.

(3) Bloch, Bruno: Das Pigment. Handbuch d. Haut. u. Geschlechtskrankh. hg. v. J. Jadassohn. Berlin, Julius Springer, 1927.

(4) Bloch, Bruno: Cancers and precancerous affections from the dermatological viewpoint. Cancer Rev., **7:** 65–98. 1932.

(5) Dalla Favera, G. B.: Ein Beiträg zur Kenntnis der Pigmentnaevi. Beitr. z. path. Anat. u. z. allg. Path., **43:** 43–88. 1908.

(6) Darier, J.: Quoted by Bloch.[4]

(7) Demiéville, Paul: Ueber Pigmentflecke der Haut. Virchow's Arch. f. path. Anat. u. Physiol., **81:** 333–354. 1880.

(8) Ehrmann, S.: Das melanotische Pigment und die pigmentbildenden Zellen des Menshen und der Wirbeltiere in ihrer entwicklung. Cassel, T. G. Fischer and Co., 1896, 80 pp.

(9) Ewing, James: Neoplastic diseases. Philadelphia, W. B. Saunders Co., 1919, pp. 157–158; 849–868.

(10) Foot, N. C.: Concerning the histology of melanoma. Am. Jour. Path., **8:** 309–319. 1932.

(11) Foot, N. C.: Concerning the histology of melanoma. II. With special consideration as to the nervous elements of the tumor. Am. Jour. Path., **8:** 321–327. 1932.

(12) GILCHRIST, T. C.: Are malignant growths arising from pigmented moles of a carcinomatous or of a sarcomatous nature? Report of two cases (one in a negro) with a study of the histogenesis of pigmented moles. Jour. Cutan. Dis., **17:** 117–131. 1899.

(13) JADASSOHN, J.: Beiträge zur Kenntnis der Naevi. Vrtljschr. f. Dermat. u. Syph., **15:** 917–952. 1888.

(14) KROMAYER, E.: Zur Histogenese der weichen Hautnaevi, Metaplasie von Epithel zu Bindegewebe. Dermat. Ztschr., **3:** 263–275. 1896.

(15) KROMPECHER, E.: Der Bazalzellenkrebs. Jena, G. Fischer, 1903, 279 pp.

(16) LANGERHANS, PAUL: Ueber die Nerven der menschlichen Haut. Virchow's Arch. f. path. Anat. u. Physiol., **44:** 325–337. 1868.

(17) LUBARSCH: Quoted by Ewing.

(18) MACCALLUM, W. G.: A text-book of pathology. Ed. 3, Philadelphia, W. B. Saunders Co., 1924, pp. 989–991.

(19) MARCHAND: Ueber die Beziehungen der pathologischen Anatomie zur Entwicklungsgeschichte, besonders zur Keimblattlehre. Verhandl. d. deutsch. path. Gesellsch., 38–105. 1900.

(20) MASSON, M. P.: Les cellules de Langerhans. Leur rôle dans les échanges dermoépidermique. Bull. Soc. franç. de dermat. et de syph., **28:** 7–13. 1921.

(21) MASSON, M. P.: Les naevi pigmentaires, tumeurs nerveuses. Ann. d'anat. pathol. med.-chir., **3:** 417–453; 657–696. 1926.

(22) MASSON, P.: Giant neuro-naevus of the hairy scalp. Ann. Surg., **93:** 218–222. 1931.

(23) MIESCHER, G.: Ein Beiträg zur epithelialen Genese der Malignen Melanome der Haut. Centralbl. f. allg. Path. u. path. Anat., **30:** 353. 1919.

(24) VON RECKLINHAUSEN, F.: Ueber die multiplen Fibrome der Haut, und ihre Beziehung zu den multiplen Neuromen. Berlin, A. Hirschwald, 1882, 144 pp.

(25) RIBBERT, H.: Ueber das Melanosarkom. Beitr. z. path. Anat. u. z. allg. Path., **21:** 471–499. 1897.

(26) RIBBERT, HUGO: Geschwülstlehre für Ärzte und Studierende. Bonn, F. Cohen, 1904, p. 255.

(27) SIMON, THEODOR: Ueber Nerven-Naevi. Arch. Dermat. u. Syph., **4:** 24–35. 1872.

(28) SOLDAN: Ueber die Beziehungen der Pigmentmäler zur Neurofibromatose. Arch. f. klin. Chir., **59:** 261–296. 1899.

(29) UNNA, P. G.: Zur epithelialen Abkunft der Naevuszellen. Virchow's Arch. f. path. Anat. u. Physiol., **143:** 224. 1896.

(30) UNNA, P. G.: Histopathology of the diseases of the skin. New York, Macmillan and Co., 1896, pp. 1126–1149.

(31) VIRCHOW, R. L. K.: Die Krankhaften Geschwulste. Berlin, A. Hirschwald, vols. 1 to 3, 1863–1867.

A FLASK FOR DILUTING ANTIGENS USED IN SERUM-TESTS FOR SYPHILIS

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The need for a simple, accurate method of diluting an alcoholic solution of lipoids with a salt solution, for use as antigen in serologic tests for syphilis, has been recognized for many years. The importance of a proper method of dilution lies in the fact that any given amount of antigen, if too slowly diluted with the correct amount of salt solution, will produce positive reactions with the serums of certain non-syphilitic persons. If too rapidly diluted with the same amount of salt solution it will produce negative reactions with the serums in certain cases of syphilis. This phenomenon is especially troublesome in the more sensitive flocculation reactions of which the Hinton test is an example.

I have had an excellent opportunity to observe the effect of variations in the rate of dilution of indicators (antigens) used in the Hinton test[1] in three laboratories under my direction. In each laboratory the method of executing the test and of mixing the reagents was based on the same instructions and the results, therefore, should have been nearly identical. Nevertheless, the indicators in each of the three laboratories differed from one another in gross appearance and the sensitiveness of the tests in the respective laboratories varied correspondingly. The differences in the indicators prepared in these laboratories grew out of failure to dilute all indicators in precisely the same way. One of the reasons for this can be readily seen if one considers that when salt solution is added, by means of a pipette, to an alcoholic solution of antigen, the rate of dilution will vary with the amount of indicator prepared at one time. For example, if one uses 1 cc. of the extract on a given day, and adds to it 0.8 cc. of 5 per cent salt solution,

drop by drop (a drop containing approximately 0.05 cc.), the ratio of dilution starts at 1 to 20; if on the other hand, 5 cc. of the alcoholic extract is required for a given day's tests, and one adds 4 cc. of 5 per cent salt solution, with the same pipette, drop by drop, the ratio of dilution begins at 1 to 100, or five times as great as when only 1 cc. of the extract is employed. The inadequacy

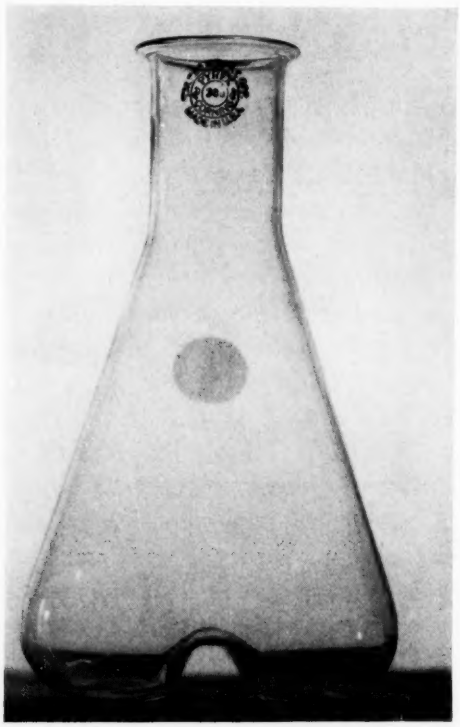

FIG. 1. INVERTED V-SHAPED PARTITION ON BOTTOM OF ORDINARY ERLENMEYER FLASK

of the drop-method is further accentuated by the fact that a given technician can not at all times equally space the intervals between the drops. Furthermore, each technician will develop a speed of dilution that is individual, in spite of the most explicit directions. Because of these factors, the rate of admixing by any drop-method will vary and will correspondingly change the turbid-

ity and opalescence of the indicator, and, likewise, will affect the sensitiveness of this reagent.

In order to overcome these difficulties, I have recently modified the shape of an ordinary Erlenmeyer flask (fig. 1) by having an inverted V-shaped partition blown in its bottom. By this V-shaped partition two semicircular compartments are produced, each of which should hold from 3 to 5 cc. in flasks of 25 to 150 cc. capacity, while in the larger flasks (150 to 500 cc.) each of the two compartments should hold from 8 to 10 cc. For the preparation of 30 cc. of glycerinated Hinton indicator, a 125 cc. flask so modified is of suitable size, while a 500 cc. flask of the same description is suitable for the preparation of 300 cc.* Flasks of this type are not listed in any of the catalogs. I have had my flasks especially modified for this purpose by Macalaster Bicknell Company, of Cambridge, Massachusetts.

When a Hinton indicator is to be prepared in this flask, the alcoholic extract should be pipetted with great care into only one of the semicircular compartments; and the proper amount of salt solution into only the other. The admixture should be made by shaking the flask quickly from side to side so that the lipoids pass rapidly from one compartment to the other. This will effect a complete mixture almost instantaneously. By the use of this flask 30, 60, 90, 120, and up to 210 cc. of glycerinated heart indicator have been prepared repeatedly, each mixture possessing the same turbidity and opalescence and likewise giving the same reactions with the same serums. Controls, consisting of separate preparations of 30, then 90, and finally 210 cc. of the same indicator, carefully mixed in ordinary Erlenmeyer flasks, showed slight but definite differences in gross appearance. The use of the modified flask for diluting Hinton indicator has simplified the technique of this test. Since indicators of almost uniform sensitiveness can now be prepared by this method of dilution, the necessity of testing simultaneously with muscle extract has been eliminated from the Hinton test. Furthermore, the suspensoid obtained by dilution in this way has made it possible to

* For the preparation of antigen for the Kahn test, we have used a 25 cc. flask modified in the same way.

consider the slightest granularity as a precipitate, which signifies a positive reaction in the Hinton test.

Other simplifications of the Hinton test which have been made possible by the use of the modified flask are described in the third modification of the test.[2]

REFERENCES

(1) Hinton, Wm. A. and Arthur Berk: The Hinton glycerol cholesterol reaction for syphilis. Second modification. New England Jour. Med., **202:** 1054–1059. 1930.

(2) Hinton, Wm. A.: Hinton test for syphilis. Third modification. Jour. Lab. and Clin. Med., **18:** 198–205. 1932.

THE LABORATORY DIAGNOSIS OF TUBERCULOUS MENINGITIS*

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The differential diagnosis of tuberculous meningitis from suppurative meningitis, poliomyelities in the preparalytic stage, encephalitis, brain abscess or subdural abscess, and syphilitic meningitis often rests on the examination of the cerebrospinal fluid. The suppurative meningitides, as a rule, can be ruled out because of the high cell count, chiefly polymorphonuclears, and by finding the causative organisms in smears or cultures. Tuberculous fluids with high counts practically always contain large numbers of easily demonstrated bacilli, provided the observer takes the time to stain for tubercle bacilli when no other organisms are found in smears stained by other stains. The Wassermann test should rule out syphilis unless complicated by one of the other diseases. The other conditions are more difficult, since on gross examination the fluids show little differences. The demonstration of tubercle bacilli in smears from tuberculous fluids according to Hemenway[5] should be possible in practically 100 per cent of cases. However, these excellent results are not duplicated in many laboratories, perhaps largely due to insufficient time given to the search.

In cases in which no bacilli are found the variations in the cell counts, globulin, gold curve, et cetera are not sufficient in themselves to differentiate between the various diseases mentioned above. Considerable aid, however, can be obtained by determining the glucose and chloride content. Giordano[4] reviewed the pertinent literature fairly completely on the question of glucose, and stressed the fact that tuberculous fluids showed a marked

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6–9, 1932.

reduction in sugar content, averaging 31 mgm. as compared with a normal average of 73 mgm. per 100 cc. of fluid. Fremont-Smith[3], Stowe[10], Kolmer[6] and Boyd[1] reported the same findings and the latter pointed out that in poliomyelitis, encephalitis and brain abscess there was no decrease in sugar.

The value of chloride determinations has not been stressed sufficiently in recent textbooks on medicine and laboratory technique. Kolmer, however, stated that tuberculous fluids show a distinct decrease in chlorides, 500 to 700 mgm., as compared with normal values of 720 to 750 mgm. per 100 cc. Neal and Esslemont[8], Pitfield[9], Levinson[7], Fowweather[2], Boyd and Fremont-Smith agreed with this finding and the latter two authors stated there was no such decrease in poliomyelitis, encephalitis and brain abscess.

This study is based on fluids obtained chiefly at the Buffalo City Hospital with a smaller number from the Buffalo General Hospital and the Pasadena Hospital. There were 147 fluids from eighty-seven cases of tuberculous meningitis, nineteen fluids from sixteen cases of poliomyelitis, seventeen fluids from eight cases of encephalitis, and thirteen fluids from eleven selected cases of severe acute meningeal syphilis. All the fluids reported as tuberculous were proved by either smear, guinea pig inoculation or autopsy. All cases proved fatal within nineteen days after onset of the first symptoms referable to the nervous system. Most of the poliomyelitis cases were severe. Seven out of sixteen died of ascending paralysis. The encephalitis patients, eight in all, lived. These included both idiopathic lethargica cases and a few cases following respiratory infections. The eleven syphilis cases included are selected cases of active syphilitic meningitis showing marked symptoms. Until lumbar puncture was done and the patients studied carefully, the type of meningitis was in doubt. In all these the Wassermann reaction was positive. Three died and were proved at autopsy, and eight recovered under specific therapy.

All of the fluids, whether tuberculous or not, showed a slight frosted-glass appearance when the cells were slightly increased, and were definitely opalescent when the cells were increased to 500

or more per cubic millimeter. The teaching that the fluids in the above conditions are clear is far from the truth if the fluids are examined in the proper light against a dark background.

On standing overnight, or even for shorter periods in the case of fluids with higher cell counts, web formation was practically constant in the tuberculous fluids. Only a few with very low counts failed to show this characteristic phenomenon. Most of the fluids in the other conditions listed showed some fibrin formation, but the typical pine tree web of tuberculosis was only rarely seen in other conditions.

TABLE 1

CELL COUNTS IN SPINAL FLUIDS

(Column headings denote cell counts per c.mm.)

TYPE	NUMBER FLUIDS	0–50	51–100	101–150	151–200	201–250	251–300	301–400	401–500	501–700	701–1,000	ABOVE 1,000	AVERAGE
Normal	100	100	0	0	0	0	0	0	0	0	0	0	2
Tuberculosis	116	14	15	18	23	9	13	10	7	3	1	3*	209†
Poliomyelitis	15	8	2	2	1	0	0	1	0	0	0	1**	79
Encephalitis	16	4	5	3	0	2	0	0	0	0	1	1**	144†
Syphilis	13	2	3	1	0	0	1	1	1	2	1	1	313

* Intraspinal serum in two cases.

† Serum treated cases excluded.

** Intraspinal serum.

Increased globulin as measured by the Pandy phenol and Ross-Jones ammonium sulphate methods and recorded as from 1+ to 4+ was noted in all the tuberculous and syphilitic fluids, averaging 2+ to 3+, whereas in the poliomyelitis and encephalitis fluid with corresponding cell counts the globulin averaged 1+ with many showing only a trace or heavy trace.

The results of the cell counts can best be seen in table 1. In one case of tuberculosis a cell count of three, and four days later of ten cells was observed. The results of guinea pig inoculation, decrease of sugar and chlorides and also autopsy findings proved the diagnosis correct. The average count in the whole tubercu-

losis series, exclusive of two counts over 1000 due to the irritation produced by administration of antimeningococcus serum before admission to the hospital, was 209 cells compared with seventy-nine in the poliomyelitis fluids and 144 for the encephalitis patients. Four fluids of the tuberculosis cases had counts from 500 to 1000 and one case showed 1120 cells. In these fluids the tubercle bacilli were abundant, and the cells were largely polymorphonuclears. The high counts in the syphilis fluids, including one of the 1109 cells in a patient who recovered, represented an extreme cellular response to a very active infection, and were in harmony with the clinical picture of severe meningitis shown by these patients.

TABLE 2

POLYMORPHONUCLEARS IN SPINAL FLUID

(Column headings denote number of cells per c.mm.)

TYPE	NUMBER FLUIDS	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	AVERAGE
Normal	100	100	0	0	0	0	0	0	0	0	0	0
Tuberculosis	130	69	6	6	11	12	9	2	6	9*	0	24
Poliomyelitis	17	13	1	0	1	1	0	0	0	1*	0	17
Encephalitis	15	9	0	0	1	2	0	0	1*	2*	0	10
Syphilis	11	9	0	0	0	0	0	0	0	2	0	18

* Under tuberculosis, 2 cases had intraspinal serum (meningococcus). Under poliomyelitis and encephalitis, intraspinal serum.

Differential counts, table 2, showed on the average a high polymorphonuclear count in the fluids showing high total counts. In all types the lymphocyte was the usually predominant cell, but no matter what the infective agent, if the infection was hyperacute, polymorphonuclears were increased. Seven fluids from patients with tuberculosis, not given any serum intraspinally, showed 90 per cent of the cells to be polymorphonuclears, and twenty-four out of 130 fluids showed 50 per cent or more polymorphonuclears. On the average the poliomyelitis and encephalitis fluids showed less, about two-thirds showing less than 10 per cent. In two syphilitic fluids from a colored patient, in which the total cell counts were 450 and 144 respectively, 90 per cent

polymorphonuclears were found. A 4+ Wassermann reaction and autopsy revealed the syphilitic nature of the meningitis.

Tubercle bacilli were demonstrated in smears in forty out of fifty cases (80 per cent), and fifty-nine out of eighty-five fluids after November 1927, when the examination was made by one of us (A. G. F.) and not left to routine laboratory procedure which gave eighteen out of thirty-seven cases positive from 1924 to 1927. (see table 3). The best smears were obtained from fresh fluids before web formation had begun. The fluids were all thoroughly centrifuged twenty to thirty minutes or more, and supernatant fluids poured off completely and the tubes never allowed to be

TABLE 3

TUBERCLE BACILLI IN SPINAL FLUIDS

	NUMBER CASES	NUMBER FLUIDS	POSITIVE SMEAR		NEGATIVE SMEAR		UNSATISFACTORY FLUIDS
			Cases	Fluids	Cases	Fluids	
1924–Nov. 1927	37	58	18	23	19	34	1
Nov. 1927–Jan. 1932	50	85	40	59	10	21	5
Total	87	143	58	82	29	55	6
Children	36	61	23	39	13	22	0
Adults	51	82	35	43	16	33	6

White *63*. Black *21*. Japanese *1*. Indian *2*.

tipped back past the horizontal in order to keep the sediment as concentrated as possible. The scummy sediments were picked up by means of capillary pipettes and smeared on warm slides and then stained by the Ziehl-Neelsen method, using a pale blue counterstain or occasionally picric acid. When web formation was present the web was teased onto a slide and spread as evenly as possible and to the same slide centrifugate of the rest of the fluid was added as above. We found the method of sedimentation depending on alcoholic precipitation of proteins not as satisfactory as the above methods. The alcohol method requires more fluid, the supernatant fluid is rendered useless for chemical tests, and the fluid is rendered sterile so that the material is lost

in case guinea-pig inoculation is to be resorted to. We were unable to obtain 100 per cent of positive tubercle bacilli findings as reported by some, for example Hemenway, but possibly our results would have been better had we had sufficient time to study smears over thirty minutes each, which must be done to give such perfect results. We did not test thoroughly the reputed advantage of examining the last bit of fluid drawn when enough fluid is removed to reduce spinal pressure to normal, but the idea seems quite plausible.

Cultures of small amounts of sediment of fluids were occasionally made on Corper's potato cylinder medium. Positive results were obtained with twelve out of fourteen fluids in which bacilli were found, and six out of eighteen in which none were found. When liberal amounts of sediment were on hand, 100 per cent positive cultures were obtained. Nine cultures were typed by Dr. Esmond Long and all found to be human strains.

The form of the colloidal gold curve was practically the same in the vast majority of the tuberculous fluids, the peak of the curve being about the 6th or 7th tube, the average reading being about 0001233210. No constancy was found in the poliomyelitis or encephalitis fluids. A few showed the same type of curve as the tuberculous, while others showed most precipitation in the luetic zone. The same occurred in the syphilitic fluids, the particular group of acute cases studied showing vastly different gold curves, varying from the usual curve seen in the average meningo-vascular neurosyphilitic.

Striking differential diagnostic evidence was obtained by quantitative determination of sugar and chlorides. These were made on fresh fluids only, by the techniques used at the time for blood chemistry, Meyer's modification of the Lewis-Benedict picric acid method for sugar and Van Slyke and Donleavy or Whitehorn method for chlorides. The fluids used as "normal" controls were obtained from consecutive fluids of ambulant patients tapped at various hours of the day, chiefly syphilitic patients given routine lumbar punctures following two courses of salvarsan. The effect of slight increase of sugar following meals may explain a few of the high sugar readings. None of these fluids showed any

increase of cells or globulin, change in the gold curve or serologic evidence of any nervous system involvement.

Our results in the various diseases (table 4) under discussion were similar to those of others, especially Giordano and Boyd. Tuberculous fluids practically uniformly showed a moderate to marked reduction in sugar. The average value was 36 mgm. per 100 cc., compared with the normal of 73 mgm., 84 per cent of the readings being below 45 mgm. All four cases showing sugar above 66 mgm. had been given intravenous glucose. No attempt was made to correlate the duration of the disease with the glucose concentration. However, in most cases in which repeated

TABLE 4

SUGAR CONTENT OF SPINAL FLUIDS

(Column headings denote mg. per 100 cc.)

TYPE	NUMBER FLUIDS	15–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–90	91–100	OVER 100	AVERAGE
Normal	35	0	0	0	0	0	0	2	4	3	4	7	2	6	5	2	73
Tuberculosis	84	19	9	13	14	16	2	3	3	1	2*	0	0	2*	0	0	36**
Poliomyelitis	14	0	0	0	0	0	0	1	2	2	2	2	0	3	1	1	75
Encephalitis	15	0	0	0	1	0	1	2	0	1	0	0	1	5	3	1	76
Syphilis	12	0	0	1*	0	0	0	3	2	1	1	0	2	2	0	0	63

* Intravenous glucose.

** Excluding cases given intravenous glucose.

determinations were done there was a slight to moderate drop in the sugar values as the disease progressed. Also on the average the fluids with the highest cell counts showed the lowest sugar content. The fluids from the poliomyelitis and encephalitis cases showed practically normal average sugar content, although some were decreased slightly, while others showed an increase. One patient with encephalitis who recovered showed 40 mgm. sugar and four days later 48 mgm. and a slight decrease in chlorides, 640 mgm. This is the only patient in the entire group or in our entire experience showing a decrease in both sugar and chlorides who did not have a tuberculous meningitis. One specimen in a case of syphilis showed a sugar of 35 mg. The

cell count in this patient was 742 and the chloride was normal. The other specimens from patients with syphilis averaged only slightly less than normal sugar.

Twenty-four normal fluids were studied fresh and then nineteen to twenty-four hours later without any preservative added. The average drop in sugar content was 4 mgm. per 100 cc. Three fluids showed a decrease of 10 mgm. and none more. Consequently we feel that sugar estimates on fluids drawn during the night are of some value if done during the following day, although all the figures in the above tables were obtained on fresh fluids.

Our experience with chloride determinations are practically in accord with other workers, especially Boyd, who stated that he

TABLE 5

CHLORIDE CONTENT OF SPINAL FLUIDS

(Column headings denote mg. per 100 cc.)

TYPE	NUMBER FLUIDS	BELOW 500	501–550	551–600	601–650	651–700	701–750	751–1,000	AVERAGE
Normal	31	0	0	0	1	9	20	1	708
Tuberculosis	65	0	10	21	27	6	1	0	603
Poliomyelitis	8	0	0	0	1	3	3	1	718
Encephalitis	12	0	0	0	2	4	5	1	700
Syphilis	9	0	0	0	0	6	2	1	697

had never seen a reading as low as 600 mgm. per 100 cc. in any other condition than tuberculosis, and that he has very seldom seen a case of tuberculous meningitis with a chloride reading of over 650 mgm. Fifty-eight out of sixty-five tuberculous fluids (table 5) showed values below 650 mgm. The fluids from cases of encephalitis averaged about normal, those in poliomyelitis slightly above, and those in syphilis slightly less, but all of these fluids showed chloride contents within the range shown by normal fluids.

From the clinical standpoint it is interesting that practically all the patients in the tuberculosis group who were in the hospital long enough for study showed evidence by clinical, x-ray, and postmortem findings of generalized miliary tuberculosis. Fifty-

one patients were adults, largely derived from the tuberculosis division of 200 beds in the Buffalo City Hospital. Of these the primary source of the infection in thirty-five cases was in the lungs, six in bones, five in hilum nodes, and one case each in the kidney, tubes and mesenteric nodes. Four patients were less than six months old, and twenty-one patients were under four years of age.

SUMMARY AND CONCLUSIONS

In the diagnosis of tuberculous meningitis from cerebrospinal fluid, the findings of tubercle bacilli on smear or culture or the production of tuberculosis in a guinea pig injected with the fluid is conclusive evidence of the disease. However, by the last two means the diagnosis can rarely, if ever, be made before the patient is dead. Smear examination if done on repeated samples should reveal bacilli in 80 to 100 per cent, depending on the technique and persistence of the examiner. But in the absence of a positive smear the following findings, especially when they are all present, (and they usually are) should warrant the tentative diagnosis of tuberculous meningitis: (1) Increased cell count, averaging about 200, producing a slight ground glass appearance in the fluid; (2) Differential count usually shows marked preponderance of lymphocytes but may show a moderate number of polys in smear-negative cases; (3) strong tests for globulin (2+ to 4+); (4) the formation of an inverted pine tree web on standing for several hours or overnight; (5) colloidal gold curve showing maximum precipitation in the 6th or 7th tube; (6) the sugar content moderately or markedly reduced, averaging about 36 mgm. per 100 cc. fluid, and (7) a decrease in chlorides below 650 mgm. Especially should the association of a marked decrease in sugar accompanied with a noteworthy decrease in chlorides in fluids not showing characteristics of a suppurative meningitis be stressed as a strong presumptive sign of tuberculous meningitis.

REFERENCES

(1) Boyd, William: The pathology of internal diseases. Philadelphia, Lea & Febiger, 1931, p. 730.

(2) Fowweather, F. S.: Chlorides in the cerebrospinal fluid in cases of meningitis. Arch. Dis. Childhood, **5**: 80–82. 1930.

(3) FREMONT-SMITH, FRANK: The spinal fluid in meningitis. New England Jour. Med., **199:** 264–265. 1928.

(4) GIORDANO, A. S.: The diagnostic value of the sugar content in the cerebrospinal fluid. Jour. Lab. & Clin. Med., **12:** 858–864. 1927.

(5) HEMENWAY, J.: The constant presence of tubercle bacilli in the cerebrospinal fluid of tuberculous meningitis. Am. Jour. Dis. Childhood, **1:** 37. 1911.

(6) KOLMER, JOHN A. AND BOERNER, FRED: Approved laboratory technic. New York, London, D. Appleton & Co., 1931, p. 248.

(7) LEVINSON, A.: Cerebrospinal fluid. St. Louis, C. V. Mosby Co., 1929, p. 296.

(8) NEALE, A. V. AND ESSLEMONT, M. S.: Observations on the chloride, sugar and calcium contents of cerebrospinal fluid in children. Arch. Dis. Childhood, **3:** 243–256. 1928.

(9) PITFIELD, ROBERT L.: Tuberculous meningitis, tuberculoma, and the total chlorides in the spinal fluid. Arch. of Pediatrics, **44:** 502–507. 1927.

(10) STOWE, W. P.: The diagnostic value of the cerebrospinal fluid sugar content. Jour. Lab. and Clin. Med., **11:** 307–312. 1926.

THE ROSE BENGAL TEST OF LIVER FUNCTION*

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The common criticism of liver-function tests is that the liver is an organ of such protean activity and of such varied function that any single test fails to measure its true efficiency. This argument is not totally valid. The histological similarity of all liver cells is so complete that it is evident, more than in any other organ, that each cell carries on every function of the organ as a whole. Any functional test that will give a definite idea of the relative number of liver cells occupied at any one function, will measure quite accurately the capacity of the liver in general.

The following types of tests have been suggested for use:

(a) Nitrogen partition methods are cumbersome and have given inconstant results.

(b) Sugar metabolism tests have proved uncertain and contradictory. The best is probably the galactose excretion test of Bauer. Apparently this has been significant in acute degenerations only. So many endocrine and general metabolic factors enter into the sugar tests as to make the liver's part of the process difficult to evaluate. Quantitative determinations of blood sugar at frequent intervals in sugar tolerance tests, as recently outlined by Kerr et al.,[6] may give a much clearer insight into certain liver functions. Unfortunately their detailed blood chemical analyses are impractical for general clinical use.

(c) The icteric index determination and Van den Bergh reaction are merely tests of the bile content of the blood and cannot be classed even as liver function tests. Their greatest value is in following the development or recession of jaundice.

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(d) The cinchophen oxidation test, recently presented by Lichtman,[7] is interesting, but by his own estimation shares with the galactose test a lack of sensitiviness in chronic conditions of liver damage, such as the cirrhoses. Giving cinchophen to patients with damaged livers is obviously of questionable safety.

(e) The specific dye excretion tests, employing phenoltetrachlorthalein, brom sulphthalein or rose bengal, offer, to date, the most clear cut results. We have chosen to work with rose bengal because, as previously determined by Delprat,[2] it fulfills all the requirements of being a highly colored, non-toxic, crystalloid dye, specifically removed from the circulation by the liver. Brom sulphthalein might quite possibly be used equally well by our same technique, were the normal removal rate determined for a comparison.

We believe the technique of Rosenthal to be essentially faulty in assuming that a dosage of a 2 mgm. per kilogram of body weight will give a uniformly colored blood plasma that can be duplicated by a fixed artificial standard prepared in vitro. Obviously the blood volume per kilogram body weight varies tremendously between an obese woman of adiposogenital dystrophy type with small heart and vessels, and a lean, muscular man of less weight but larger vascular bed; and the color imparted to the blood by a fixed 2 mgm. per kilogram dose of dye must vary as much.

Our technique[4] has been published in detail recently. Briefly it consists of injecting into an arm vein, irrespective of body weight, from 5 cc. to 10 cc. of 1 per cent or 2 per cent rose bengal in saline. Between these limits, at least, the amount injected does not matter. We have duplicated results on successive days, on the same patients, with the upper and lower limits of this dosage. The higher dosage gives more easily read solutions.

Two minutes from the start of injection the "standard" sample of blood is withdrawn into a clean, dry syringe and exalated. This is an in vivo mixture of the dye with the patient's blood, automatically corrected for pigment discoloration and for blood volume. Exactly six minutes later, the second, or "unknown," blood sample is withdrawn. The only exact point in technique

required is the timing of this six minute period. Many normal control tests have determined that the fall in color in that six minutes should be fifty per cent of the "standard." The plasma is separated from the two blood samples by centrifugation, cleared of turbidity and of most of its interfering pigment color by precipitation of proteins with two volumes of acetone and recentrifugation. The resultant dye solutions are crystal clear and easily compared in the colorimeter. We have been accustomed to remove about 8 cc. of blood for each sample, using 3 cc. of plasma and 6 cc. of acetone for precipitation. If the resultant dye solutions are still yellowish from bile pigment, it has been found of value to add three drops of saturated sodium hydrate solution to each, let stand about fifteen minutes in subdued light, and recentrifugate. Pure rose colored solutions usually result.

The calculation is simple if it is remembered that the unknown should give one half the colorimeter reading of the standard for one hundred per cent function. Higher readings mean correspondingly lower function. Algebraically expressed in simplest terms:

$$200 - \frac{200\ Rs}{Ru} = \text{per cent of normal liver function}$$

where Rs is the colorimeter reading of the first (2 min.) sample and Ru of the second (8 min.) sample.

Because of the photosensitizing effect of rose bengal on the tissues, patients should be kept out of direct sunlight for a few hours after the dye is given. It is also advisable to warn them, and their nurses, that their stools will be red from the dye, lest they be alarmed at supposed intestinal hemorrhage.

Several chemically different dyes have been called rose bengal. It is essential to use an eight halogen atom dye as those with six halogen atoms are in part eliminated by the kidneys. We have used the "Special for Intravenous Use" rose bengal prepared by Coleman and Bell, chemically di-sodium-tetra-iodo-tetra-chlor-fluorescein.

RESULTS

Normal values, (85 to 110 per cent) 17 cases. (Normal pregnancy (3 cases); chronic colitis; cancer of breast with no metastases; peptic ulcer; asthma; influenzal pneumonia; chronic alcoholism (2 cases); chronic cholecystitis of mild grade (3 cases); trichiniasis; pre-eclampsia; intestinal obstruction; obstructive jaundice from scar tissue around duct (old operation). This last patient is of particular interest in showing 100 per cent liver function despite the presence of marked jaundice.

Gall-bladder disease. Rose bengal elimination was in inverse relationship to the acuteness of the disease. Two patients with mild chronic disease gave 100 per cent function. One patient with subacute disease without jaundice, 80 per cent, one patient with acute disease, unable to retain food for a week, 70 per cent. Two patients in acute attacks, with fever and leucocytosis, superimposed on years of previous attacks, gave 60 per cent function on admission and 70 and 80 per cent respectively after a few days of glucose medication and relief of symptoms. One patient with a history of two years of gall-bladder attacks, gall stones and a "hard liver" found at operation, showed 50 per cent function. One patient with complete obstruction from stone in the common duct gave 30 per cent function, rising to 100 per cent three weeks after passage of the stone spontaneously.

Toxemias of pregnancy. In three normal pregnancies the function varied from 85 to 100 per cent. One patient with pre-eclamptic toxemia of mild type showed 100 per cent, one patient with early pre-eclamptic toxemia, 80 per cent, which rose to 105 per cent in ten days after delivery. One eclamptic of severe grade showed 55 per cent function, rising to 85 per cent in ten days after cesarean. Two patients with hyperemesis gravidarum gave 55 per cent and 40 per cent of normal elimination of the dye, the former rising to 93 per cent on five days of intravenous glucose and relief of vomiting.

Cirrhoses. Low values were uniformly obtained. The patients with typical Laennec type of four and five years duration gave 32 per cent and 33 per cent functions, one patient with Hanot type

showed 45 per cent, one following cinchophen hepatitis of seven months resolution, with recent disappearance of jaundice, 35 per cent function. Residual cirrhosis was doubtless present in this patient.

Alcoholism—chronic. Values here were 100, 90, 80 and 42 per cent. The last patient entered the hospital in his fourth attack of delirium tremons within two years. Alcoholic cirrhosis was evidenced by an enlarged liver and jaundice. A month later the rose bengal elimination had only increased to 48 per cent, while the jaundice was still present. This patient died about a month after the second test.

Carcinoma (secondary.) Three patients were studied. Two, with secondary cancer of the breast, had 78 and 80 per cent functions. The third, with liver metastases secondary to carcinoma of the gall-bladder, entered emaciated and starved, with 40 per cent function which rose to 70 per cent after five days of intensive glucose therapy by mouth and vein.

Diffuse hepatic damage. In addition to the patients with hyperemesis gravidarum mentioned, two are of interest. One with severe peritonitis following a ruptured appendix showed 60 per cent dye function. The other, entering with 67 per cent function and liver abscess of several months duration following peritonitis, had a slight rise to 70 per cent function following surgical drainage of the abscess, but failed to maintain clinical improvement. A month later, twenty-four hours before death, the rose bengal was 57 per cent. Post-mortem examination of the liver showed additional abscesses and severe damage, microscopically, to the liver cells between the abscessed areas.

These results accord quite closely with those previously reported,[3,6,5] and we believe show very close correlation between the results of the test and the extent of the liver cell damage, numerically and structurally.

REFERENCES

(1) Althausen, T. L., Gunther, Lewis, Lagen, J. B., and Kerr, W. J.: Modification of the dextrose tolerance test as an index of metabolic activity of the liver. Arch. Int. Med., **46:** 482–493. 1930.

(2) Delprat, G. D.: Studies on liver function. Arch. Int. Med., **32:** 401–410. 1923.

(3) DELPRAT, G. D., EPSTEIN, N. N., AND KERR, W. J.: A new liver function test. Arch. Int. Med., **34:** 533–541. 1924.

(4) DELPRAT, G. D., AND STOWE, W. P.: The rose bengal test for liver function. Jour. Lab. & Clin. Med., **16:** 923–925. 1931.

(5) EPSTEIN, N. N., DELPRAT, G. D., AND KERR, W. J.: The rose bengal test for liver function—further studies. Jour. Am. Med. Assn., **88:** 1619–1623. 1927.

(6) KERR, W. J., DELPRAT, G. D., EPSTEIN, N. N., AND DUNIEVITZ, MAX: The rose bengal test for liver function. Jour. Am. Med. Assn., **85:** 942–946. 1925.

(7) LICHTMAN, S. S.: Cinchophen oxidation test of function of the hepatic cells. Arch. Int. Med., **48:** 98–125. 1931.

THE PRESENT STATUS OF THE SEROLOGICAL DIAGNOSIS OF SYPHILIS, WITH SPECIAL REFERENCE TO BASIC PRINCIPLES*

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Additions to the overburdened literature on the serology of syphilis demand temerity and necessitate some attempt at justification. The present discussion arises from the belief that the renewed flood of contributions within recent years has tended to produce a rather paradoxical situation in that recent developments have clouded rather than clarified the serological study of syphilis which at present appears struggling between the Scylla of laboratory incertitude, on the one hand, and the Charybdis of clinical chaos, on the other. The impartial observer, especially if his angle of view be sufficiently comprehensive, cannot fail to find support for such a contention nor to appreciate also that the discussion has greatly narrowed in scope.

We do not appear to be concerned solely with syphilis as a diagnostic and therapeutic problem of paramount importance to patient, clinician, and community; nor primarily with the actual or relative value and utility of the various laboratory procedures available for its study. On the contrary, the discussion has had an insidious but perceptible drift into a much narrower circle and now appears to be transformed into a struggle for survival or annihilation, a series of individual combats, as it were, the purpose of which would seem to be the selection by elimination of a serological champion who, surrounded by prostrate foes, shall dominate the arena.

The contest now appears to have a double objective, based

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first, upon a clear-cut endeavor by the proponents of precipitation tests to eliminate the complement-fixation reaction from participation in the study of syphilis, and, second, upon what may soon assume the appearance of a squabble as to which precipitation reaction shall be the sole serological procedure to be used. This is stating the proposition with more bluntness than grace but that some such situation is at least in the making, if it does not already exist, can hardly be denied.

It seems, therefore, not only justifiable but profitable as well to regard the subject anew from a general rather than a particularized standpoint; not merely as proponents of this or opponents of that contention, but rather in an endeavor to see the problem as a whole with sufficient perspective to assign a true rather than an artificial importance to its many and varied phases.

This is the end, to be attempted if not achieved, of the present discussion in the pursuit of which it is profitable to reduce the subject, if possible, to basic, elemental principles, fundamental propositions, as it were, of paramount importance from which all else may be derived from logical processes.

THE FIRST PRINCIPLE

It has long been recognized that syphilis, in regard to both its recognition and control, presents one of the most intricate and puzzling problems in the entire field of medicine. Because of the tremendous potentialities involved and the far-reaching and sometimes disastrous possibilities of its aftermath, the diagnosis of syphilis is of paramount importance to the patient; for the same reason, so gravid with disaster is an erroneous diagnosis that every precaution should be evoked to prevent a missed diagnosis on the one hand as well as an erroneous assumption of the presence of this disease on the other.

It is essential, therefore, in any consideration of this infection, that the interests of the patient must be always predominant, and it may be set down as a first and highly important principle, that *the diagnosis of syphilis, however achieved, should be surrounded by every possible safeguard*, regardless of the time, the labor, the expense, or the minutia involved.

THE SECOND PRINCIPLE

One cannot consider the problem of syphilis, even in the most cursory manner, without realizing, that the recognition of any specific pathological entity as the underlying mechanism responsible for symptomatic manifestations is less often, and undoubtedly with less certainty, achieved by inspiration than by a thorough and painstaking consideration of all the possibilities which come within the knowledge and recollection of the observer. Nor can it be overlooked that the number and diversity of these possibilities is in direct proportion to his ability to detect in the patient all the manifestations of disease, no matter how cryptic or how minor, and to attempt their correlation with as many possibilities as the broadness of his information includes.

Particularly is this true and of essential importance in syphilis because of the ability of this disease to simulate others and to manifest itself, especially in its congenital, latent, concealed, and later stages, in cryptic and even bizarre fashions the true evaluation of which may well tax the skill and acumen of the clinician to the uttermost.

Upon these well-recognized but not always equally well-emphasized factors is based the maxim that it is well "to be quick to suspect syphilis but slow to diagnose it," which is only another way of emphasizing the many, diverse, and obscure ways in which the disease may first present itself to the physician.

It may well be accepted therefore as a second essential principle applicable to this tremendous problem, that *the diagnosis of syphilis should be based upon a careful, well-balanced consideration of all the data, however obtained, rather than predicated solely upon one or two isolated particularized facts or findings.*

THE THIRD PRINCIPLE

The deeper one delves into the study of disease in general and of syphilis in particular, the more obvious it becomes that in the detection and measurement of its manifestations a variety of methods must often be called into play, among them those embodied in the varied procedures of the clinical laboratory.

In few conditions is this more often of importance than in syphilis, for the accumulated experience of the past has demonstrated beyond cavil that the problem of syphilis may be and often is insolvable by all the usual avenues of clinical approach, no matter how well applied.

Past experience has demonstrated also beyond any need of further contention that of all the available avenues of laboratory approach to the study of syphilis, none are more generally applicable nor more delicate and reliable than the serological.

In view of these facts, then, as a third principle it may be stated that *a careful and intelligent study of syphilis is impossible without constant resource to laboratory avenues of investigation, especially serological studies.*

The proposition thus stated is now practically self-evident and has become well established through the numerous, varied, and cumulative investigations of innumerable workers and there is no dispute of the contention that, when wisely applied and intelligently interpreted, serological studies are of inestimable value in syphilis, just as they may be productive of incalculable harm unless properly safeguarded.

It is necessary that these elementary principles be reiterated and emphasized because an unfortunate tendency of modern times, arising from modern developments and refinements in the procedures of the laboratory, has been the development of an attitude which ascribes to laboratory procedures a paramount rather than an ancillary importance in the study of the patient.

In other words, many physicians have shown a tendency to make the laboratory investigations a prime rather than a complemental factor, and to think of them and utilize them as "tests" the results of which may be taken as indicating the presence or absence of disease. This is an erroneous as well as a dangerous conception, for the true function of laboratory procedures is simply and solely that of methods for the detection and measurements of response to varied stimuli, and as such they constitute simply one, though a very important and often indispensable, phase of the examination of the patient. It is only when thus regarded and so utilized that laboratory procedures can be wisely

and safely applied, and it is to this end that clinical pathologists have loudly decried undue and blind reliance upon them.

THE FOURTH PRINCIPLE

It is, therefore, both timely and essential, to accept as a fourth principle that *laboratory procedures must be regarded solely as constituting a single phase in the examination of the patient;* as furnishing means for the detection and measurement of the response to varied and not always pathological stimuli, recognizing that their real and greatest value rests upon their intelligent interpretation from which the nature and often the degree of stimulus responsible for their results may frequently be derived by inferential reduction.

To the cursory or superficial observer it would seem unnecessary to repeat and re-emphasize dicta apparently so trite as those embodied in the principles above stated. But the experience of serologists and syphilographers furnishes many and tragic examples of their neglect and leaves no room for doubt that many individuals are stigmatized as syphilitic and condemned to years of treatment and observation on the basis of what, after all, is really only a piece of paper inscribed with the symbol "plus four reaction," while in many others the spirochetes pursue the unmolested and even tenor of their malevolent way because a similar piece of paper bears the magic inscription "negative."

Serological methods constituted the first as well as one of the greatest advances made in the study of syphilis; but they cannot and must not be regarded as unimpeachable and infallible, nor can they escape compliance with that law of common sense which compels recognition of the fact that, in the last analysis, it is not the test but its interpretation which is of significance.

Accepting, then, serological procedures as indispensable in the study of syphilis, serologist, syphilographer, and clinician must next consider and establish, as definitely as possible, their relative value and reliability, and, what is of essential importance, must determine how their practical utilization may be best safeguarded against malapplication and malinterpretation.

It is obvious that so long as there are cryptic and latent stages

of syphilis in which no amount of clinical skill, experience, or acumen suffices to discover the presence of the disease nor to establish evidences of its eradication or control, serological procedures constitute a most valuable and important clinical weapon of attack.

It is equally obvious also, that the present tendency is to narrow the scope of serological investigations, and to select one method in preference to all others. To this proposition many appear to be more or less committed while others, approaching the other extreme, would combine the use of all available methods, and still others, seeking the middle path, would check one procedure by another varying in principle or technic.

While variations in technic are daily becoming more numerous —many, however, being but exceedingly minor deviations from established methods, so minor as scarcely to justify the naming of a "new" test, serological procedures in syphilis, whatever their nomenclature, fall into one of two groups and are based upon somewhat similar if not identical mechanisms.

On the one hand, are those dependent upon the ability of syphilitic serum to fix complement in the presence of a suitable antigen, and on the other, those arising from the property of syphilitic serum producing flocculation or precipitation of a suitable colloidal suspension.

The first of these, the complement-fixation reaction, is the older and has been subjected to the most prolonged and intensive investigation to date; the second, the precipitation test, is the newer and the one about which at present the most acute interest seems to center.

It is exceedingly unfortunate that the complement-fixation test is so commonly spoken of as the "Wassermann test," for the procedure as conducted today bears no resemblance to that originally described by Wassermann and has in common with it only the basic principle underlying the mechanism upon which it depends.

It is still more unfortunate, and this indeed is the source and cause of much of the clinical confusion undoubtedly existing, that to far too many clinicians a Wassermann test is a Wasser-

mann test regardless of how or by whom performed, and the results of which are accepted or discarded with little or no endeavor to determine the reliability of the method used or to estimate the competence, skill, and conscientiousness of the worker by whom it was applied.

It is, without exaggeration, both terrible and inexcusable that diagnoses should be made or discarded and treatment begun or abandoned for no better reason than the existence of a report, which too often represents almost all the investigation made to determine the presence or absence of evidence of syphilitic infection, and concerning the reliability of which but little concern is manifested, regardless of whether the reaction be one of complement-fixation or precipitation.

THE FIFTH PRINCIPLE

It should be recorded as a fifth principle, therefore, that *a joint and interlocking responsibility rests upon both serologist and clinician entitling each to demand somewhat of the other and obligating both to a joint utilization of their combined resources in the interest of the patient.*

Of the serologist it may be expected that he be truly a serologist well-grounded in the elements of this specialty and not merely a manipulative expert; that he should not be unalterably wedded to a single method but eager and willing to investigate the merits of new proposals and, when warranted, to adopt them entire or in part, and finally, that, when called upon, he should have sufficient clinical experience to correlate correctly the combined clinical and laboratory data of a particular case.

Of the clinician it may be demanded that he be sufficiently acquainted with the rationale and mechanism of serological procedures to apply them intelligently and interpret them wisely; that he realize that a positive or negative serological report signifies solely that the blood examined did or did not contain at the time of examination complement-fixing or precipitating substances and that the significance of the reaction is a matter wholly beyond the fact of its occurrence, and finally, that if he assumes the responsibility for the interpretation, he also assume the

responsibility for acquiring sufficient familiarity with the details and factors influencing the result, that he be perfectly familiar with the limitations of the test in question, and competent to evaluate properly its results.

In view of what has already been outlined it is necessary now to consider the methods properly applicable to the serological study of syphilis and from the comprehensive, intensive, critical, and even hypercritical studies of over a quarter of a century the sixth principle is readily derived.

THE SIXTH PRINCIPLE

The complement-fixation reaction, in its modern, refined, and perfected form as exemplified, for instance, by the quantitative method devised by Kolmer, constitutes not only one of the most valuable and reliable laboratory procedures at present available, but also when positive, may be regarded with confidence as the most delicate and constant single sign of syphilis.

So extensive have been the investigations upon the results of which this principle is based, and so thoroughly have they been discussed and evaluated in the voluminous literature of this subject, that there is no need to reconsider them here.

So comprehensive, likewise, have been the studies of the quantitative method of Kolmer that its status is no longer a matter of contention: If one is not willing to accept it as the best at present available, it can be and is accepted as a standard with which other methods, precipitation as well as complement-fixation, may well be compared.

My own fairly extensive experience with it has satisfied me with its delicacy, reliability, relative specificity, and clinical applicability in every respect. *But it must be said that this applies only when the method is used as described without distortion of its principles or evasion or omission of its essential minutia.*

THE SEVENTH PRINCIPLE

Modern developments in the field of serology have furnished data from which a seventh principle may be derived, based upon the fact that syphilitic serum possesses a colloidal instability as

a result of which, when brought into contact with a suitable colloidal antigenic suspension under properly controlled conditions coherence or aggregation of suspended colloidal particles occurs.

So constant is this phenomenon and so extensively has its occurrence been studied that it may now be stated as a seventh principle: *that so great is the practical specificity of precipitation tests under properly controlled conditions, that they may be accepted as valuable additions to the serological study of syphilis, and as useful if not essential adjuncts to the complement-fixation test.*

The practical value of precipitation tests in the serological study of syphilis is now definitely established by the work of Kahn, Kline, Hinton and others who have subjected these and similar methods to long, careful, and extensive study, and, as just stated, precipitation reactions have now an established standing and their usefulness is no longer a matter of dispute.

What is a matter of contention, however, is the exact status to be accorded them; whether they shall be used as adjuncts to the time-tried complement-fixation test, or whether they shall constitute a sole and sufficient criterion for the laboratory study of syphilis. Before this latter assumption can be accepted with safety and without reserve many factors must be carefully considered, among the more important of which are these: It cannot be overlooked that, regrettable and, in fact, almost inexcusable as it may be, in no small proportion of cases the serological investigation for evidence of syphilis is all too often restricted to the examination of a single specimen.

Any serological procedure to be accepted as an ideal, infallible, and final procedure the results of which shall be accepted as incontrovertible, necessitates, under the circumstances, that the following criteria shall be fulfilled: (1) It must possess an absolute specificity for the disease. (2) It must infallibly and consistently detect the presence of syphilitic reagin in any and all stages of the disease. (3) It must not give a positive reaction in the absence of syphilitic reagin. (4) It must be, to all intents and purposes, "foolproof."

It is obvious, of course, that no single procedure exists which

can meet these requirements. Nor is it possible that any such procedure will ever be devised because of factors inherent in the disease and, what is of still greater importance, the reaction of the patient to the disease.

THE EIGHTH PRINCIPLE

Until the nature of syphilitic reagin is discovered it is impossible, to devise or elaborate truly biologically specific tests for its presence.

Rather paradoxically, however, the almost incalculable amount of work which has been done in this field has established beyond question what may be accepted as an eighth principle in the field of serology, namely: *that while both the complement-fixation and the precipitation reactions are biologically non-specific, they possess, nevertheless, an extraordinary degree of practical specificity when properly performed under carefully controlled technical conditions, so much so that positive reactions are consistently encountered in only one disease other than syphilis, namely, yaws.*

It must be emphasized, however, that this can be accepted as true only under acceptable and controlled technical conditions and only when these are strictly adhered to and competently carried out. The statement cannot be applied to nor accepted of any and all of the numerous and diversified methods collectively and generally spoken of as "Wassermann tests," nor of any and all of the precipitation methods. On the contrary, it may be said with little fear of error that it applies in the strictest sense to but relatively few complement-fixation methods, among them that of Kolmer, and to but few of the precipitation methods.

It should also be emphasized, however, that there is no method of conducting complement-fixation or precipitation tests at present available, nor can it be expected that any will ever be devised, with which it will be impossible to obtain non-specific false positive reactions. This is inescapably true because false positive reactions may be so readily produced by technical errors due to carelessness, malexpertness, or manipulative ineptitude.

This fact alone renders blind reliance upon any single isolated serological reaction exceedingly dangerous unless it has been

carried out with a high degree of technical skill fortified by thorough training and supplemented by a conscientious sense of the responsibility involved.

In view of the influence of technic upon the production of nonspecific reactions, it is of great practical importance to emphasize certain technical features of both complement-fixation and precipitation tests which are of importance in this connection.

1. Much has been said of the simplicity of the precipitation as compared to the complement-fixation tests; but equal emphasis has not been laid upon the fact that the underlying mechanism of both is quite similar if not identical, and that, to this extent, one is no simpler than the other. Nor has it always been equally emphasized that, as has been amply demonstrated by practical experience, it is far easier to train a technician to conduct a complement-fixation test with technical accuracy so that the results shall be clear-cut and without question, than to train a technician to perform precipitation tests with an equally consistent degree of skill.

It is my opinion that the inherent complexity of the complement-fixation test should be its greatest safeguard in that the necessity for careful, thorough, and extensive training fortified by experience required for its intelligent performance should restrict its performance to those so trained and adapted. For it may be stated without equivocation that neither complement-fixation nor precipitation tests should be performed by those whose knowledge of their minutia is, at best, superficial.

2. Regardless of the technical skill and care exhibited by the worker, the weakly positive, indefinite, "plus-minus" reaction will always be a source of trouble and incertitude largely because of the uncontrollable factor of the personal equation which influences their determination.

It is difficult to see how the reading, classification, or interpretation of such reactions can ever be safely standardized without loss in delicacy and specificity and to this extent precipitation tests present difficulties not encountered in the complement-fixation reaction, to which, in this sense, they are inferior.

3. Perhaps the one feature of precipitation tests which has

been most clamorously extolled is the rapidity with which they may be completed.

Rapidity, however, cannot be accepted as sufficient reason for the exclusive adoption of precipitation tests unless one accepts also the necessity for precipitate haste in diagnosis; and wherein lies the necessity for instantaneous diagnosis?

It cannot be because of danger to the patient by reason of delay of a few hours or even days, because syphilis, at best, is an intractable disease and its treatment slow and tedious. It is not a disease in which life, death, or ultimate cure is a matter of days or months; efficient treatment is a matter of years. In the early case, by the time a serological reaction is detectable the disease has been systemically disseminated; in the late case, or one rediscovered years after insufficient treatment, a further delay of forty-eight to seventy-two hours is of little importance.

There should be no opposition to nor regret for whatever may be necessary in time or labor to render the diagnosis of syphilis free from error or to safeguard the treatment of this dreaded malady.

As has already been said, the ideal serological test for syphilis should be capable of detecting the presence of syphilitic reagin in any and all stages of the disease, a requirement incapable of fulfilment by any method now available or, indeed, ever to be devised. The reason is obvious: Because the production of reagin is dependent upon and in great measure proportionate to the degree of interaction between the invading spirochetes and the reacting tissues. It is not enough that spirochetes be present. They must attack the tissues and the tissues in turn must react to the attack. Unless these postulates are fulfilled the production of reagin is impossible. Even the most elementary consideration of syphilis forces the realization that spirochetes may be present and viable and yet tissue reaction, if not absent, may be so minimal that reagin production will be far below the conceivable limits of detection.

It is this proportionate relation between tissue reaction and reagin production which explains the failure to obtain positive serological reactions immediately following the initial spirochetal

invasion, during the early days following the appearance of the chancre, and in the later, latent, and inactive stages of the disease.

THE NINTH PRINCIPLE

This is also the underlying reason for what may be taken as the ninth principle applicable to the serological study of syphilis, namely, that *there is no serological procedure at present available, nor is it probable that one will ever be devised, with which a false negative reaction may not be obtained.*

Where the management of syphilis is in competent hands, false negative serological reactions are of relatively minor importance and productive of but little harm as they will be subjected to interpretation in the light of all the data pertaining to the particular case and checked by further and additional studies.

But as long as it remains true, as it is true in a definite proportion of cases, that the diagnosis of syphilis and, particularly, decisions concerning the necessity for initiation or cessation of treatment are dependent largely upon the results of infrequent and perhaps isolated serological examinations, the occurrence of false negative reactions will remain a matter of practical importance.

It must not be forgotten, also, that false negative reactions may arise from the spontaneous and sometimes marked fluctuation in reagin content which, for reasons as yet unknown, may be concomitant with any stage of the disease and any degree of activity.

This one phenomenon alone is sufficient illustration of the danger of relying upon an isolated negative reaction as indicative of the absence of syphilis or as furnishing a safe and infallible basis for the cessation of treatment.

There is still one other explanation for the occurrence of false negative reactions based upon the practical experience of serologists at large which collectively has demonstrated a selective affinity on the part of reagin for various antigens.

This is more commonly discussed in terms of antigen sensitivity and refers to the ability of antigenic suspensions to react with reagin.

That this is a peculiarity of the reagin, however, rather than of the antigenic colloidal suspension is shown by the fact that antigenic suspensions reacting with a majority of positive sera will fail to react with a particular positive serum, while the same serum will react strongly with another antigenic suspension of no better quality than the first.

While the occurrence of this phenomenon has been rendered comparatively negligible by present methods of antigen preparation giving products of great sensitivity, it has not been totally eliminated as a factor of some importance.

It is this phenomenon which is responsible in every series of parallel complement-fixation and precipitation tests, for the occurrence of a variable number of tests in which, with sera simultaneously examined, the reaction is in one case positive to the complement-fixation test and negative to the precipitation test, while in another the reverse is true. This in itself is not a disadvantage because one test may thus be used to check the other and the chance of a false negative reaction thereby reduced.

If this possibility furnishes an added reason for avoidance of undue reliance upon any single method, especially when it is to be applied to a single specimen, by the same token it may be taken as indicating very strongly the advisability of simultaneously utilizing both methods of procedure on every serum tested.

THE TENTH PRINCIPLE

So obvious does the widsom if not, indeed, the necessity for such a course appear from the numerous and cumulative studies that have been made that it may be accepted as a tenth principle in the serological study of syphilis, that *the simultaneous use of both complement-fixation and precipitation tests on every serum should be routinely practiced* that one reaction may both complement and corroborate the other and the chance of both false positive and false negative reactions be thereby minimized.

We are considering now, not the question of diagnostic interpretation of the reaction, because, as has already been emphasized this is always to be based upon a consideration of all available data and not merely of the serological data alone; but simply

whether the reaction shall be reported to the clinician as positive or negative.

It is apparent, of course, that this must be governed in large measure by the serologist's experience with the methods in question and his balanced judgment as to their comparative reliability, delicacy, and specificity.

My own course in such case is governed by my own past experience as well as by the reported experience of others. My routine complement-fixation test is the six-tube quantitative method of Kolmer and my routine precipitation test is the slide precipitation method of Kline. Personal experience with Kolmer's method, begun before its publication and fortified by its consistent use and many and varied comparative trials during the succeeding ten years, has taught me to accept it without question as a reliable standard of comparison. I consider it to be probably and with very little question one of the most, if not the most, reliable and delicate methods available and I am convinced that with this method, when carefully and exactly applied, nonspecific reactions are encountered with extreme infrequency, if, indeed, they occur at all. It is not, however, free from the occurrence of false negative reactions for the reasons already outlined.

My experience with the Kline test began with its publication in 1926 and since then it has been used by me in many thousands of tests in series with simultaneously conducted Kolmer tests. I believe the Kline test to be the equal if not the superior of the Kahn test and to possess a high degree of specificity and delicacy. It also, and for the same reasons as given above, is not without false negative reactions and, what is of greater importance, it occasionally gives false positive reactions.

Like all precipitation tests it presents difficulty in reading and evaluation of border-line, weakly-reacting sera because of the impossibility of compensating for or eliminating the personal equation in reading such reactions. "Plus-minus" reactions, I believe, should be disregarded except, perhaps, when the test is used as a control of treatment. To this extent the test is less satisfactory than the Kolmer test.

For these reasons, when the Kolmer test is positive and the

Kline test negative, I refer now to at least a +3 reaction in the first tube of the Kolmer set-up—I report the reaction as positive.

When the Kolmer test is negative and the Kline test is positive I prefer to repeat the test either with the same serum, using another Kolmer antigen, or, preferably with another specimen. Because of the occasional discordance in the results of the two tests when simultaneously conducted it has been suggested that, perhaps, it would be wise to use a number of different methods, for instance, to use the Kline, the Kahn, the Hinton and the Kolmer tests on each serum. Such a proposal has various practical and important disadvantages.

In the first place, though the above list seems formidable enough it by no means exhausts the acceptable methods now available, some of which, at least, it would be difficult to exclude fairly or on logical grounds. This proposal in turn leads to the possibility that the withdrawal of the sample for testing might become a formidable operation followed by a period of convalescence and has in it the elements of a reductio ad absurdam.

For it is obvious that in the presence of discordant results some one or more reactions must be taken as the standard of comparison from which a conclusion can be drawn. Certainly, all the reactions cannot be taken as of equal value. If they were all would not be needed. If they are not, the most reliable should suffice.

If the interpretation of the reaction as positive or negative is to be made by the serologist the practical difficulty above outlined remains, but if the reactions encountered are reported to the clinician without summation, evaluation, or conclusions, the difficulty is enormously increased, so much so that the results of the whole procedure may be nullified and serological methods greatly discredited.

For it must be constantly remembered that the bulk of serological reports are made, not to syphilographers, but to clinicians at large many of whom are not syphilographers in any sense of the word and many of whom are far from skilled in the interpretation of serological reactions. No further proof of this is

needed than the inquiries, common in the experience of any serologist, as to the meaning and significance of the term "anticomplementary reaction."

What will happen then when four reactions show varying degrees of positivity and three are negative, and the patient is anxious to hear that he never had the disease or, if he had, that he has had enough treatment?

It seems to me far safer and more satisfactory for all concerned for the serologist and clinician to be well versed in the capabilities and limitations of one acceptable complement-fixation test and one acceptable precipitation test and to use them simultaneously, than to introduce unnecessary multiplications of unnecessary complications by a multiplicity of methods.

In the present state of our knowledge of the serological aspects of syphilis if it can be considered safe, wise or even justifiable to restrict arbitrarily or dogmatically the serological study of syphilis to any one single procedure, it may be demanded, at least, that any such postulate should be confronted with a full consideration of all the fundamental principles involved which, I believe, may thus be summarized:

SUMMARY

1. The diagnosis of syphilis, however achieved, should be surrounded by every possible safeguard regardless of the time, the labor, the expense, or the minutia involved.

2. The diagnosis of syphilis should be based upon a careful and well-balanced consideration of all the data, however obtained, rather than predicated upon one or two isolated or particularized facts or findings.

3. A careful and intelligent study of syphilis is impossible without constant resource to laboratory avenues of investigation and especially serological studies.

4. Laboratory procedures must be regarded solely as constituting a single phase in the examination of the patient; as furnishing means for the detection and measurement of the response to varied and not always pathological stimuli; recognizing that their real and greatest value rests upon their intelligent interpretation

from which the nature and often the degree of stimulus responsible for their results may frequently be derived by processes of inferential deduction.

5. A joint and interlocking responsibility rests upon both serologist and clinician entitling each to demand something of the other and obligating both to a joint utilization of their combined resources in the interests of the patient.

6. The complement-fixation reaction, in its modern, refined, and perfected form, constitutes, not only one of the most valuable and reliable laboratory procedures at present available, but also may be regarded with confidence, when positive, as the most delicate and constant, single symptom of syphilis.

7. So great is the practical specificity of precipitation tests, under properly controlled conditions, that they may be accepted as valuable additions to the serological study of syphilis and as useful, if not essential, adjuncts to the complement-fixation test.

8. While both the complement-fixation and precipitation reactions are biologically nonspecific, they possess, nevertheless, when properly performed under carefully controlled technical conditions, an extraordinary degree of practical specificity, so much so that positive reactions are consistently encountered in only one disease other than syphilis, namely, yaws.

9. There is no serological procedure at present available, nor is it probable that one will ever be devised, with which a false negative reaction may not be obtained.

10. The simultaneous use of both complement-fixation tests and precipitation tests should be routinely practiced on every serum that one reaction may complement and corroborate the other and the chance of both false positive and false negative reactions be thereby minimized.

A DISCUSSION OF THE PAPERS ON SERODIAGNOSIS OF SYPHILIS BY DOCTOR KILDUFFE AND DOCTOR LEVINE*

RUTH GILBERT. The aspects of the problems concerned with the serodiagnosis of syphilis which have been presented this afternoon seem especially worthy of consideration. Possibly in no other medical field is America taking

*See the JOURNAL, **2**: 319, 1932.

the lead more definitely than in the serology of syphilis. I think that we in this country may feel justly proud of the achievements that have already been accomplished.

As was exemplified in the work presented today, the importance of delicately adjusted procedures which will give high percentages of reactions with specimens from syphilitic patients and a minimal number with those from persons without history or clinical evidence of the disease cannot be too greatly stressed. Experience with the work in a number of large laboratories in this country and more direct contact with that in New York State, leads me to believe that the chief problem at present is to secure sufficiently sensitive results. The tests widely used in the United States apparently tend to furnish very small percentages of unexplained reactions. I am sure that you have all observed that, barring those due to clerical or technical errors, the number of this type of reaction bears a distinct relation to the care, ability, and primary interest shown by the clinician in charge of the patient. With the skilled syphilologist, the number of apparently false reactions secured becomes practically nil, the majority of such results being found to occur with specimens submitted from institutions where the physicians are primarily interested in other fields of medicine. In this connection, the possibility of some drain on the vitality of the organism, such as that due to trauma, febrile or malignant disease or pregnancy, being a contributing factor in the activation of a latent, acquired, or congenital syphilis may sometimes be overlooked.

Dr. Levine and Dr. Kilduffe have stressed the importance of proper control of the work. The use of two different procedures, as recommended by them and at the League of Nations conferences, if the results are comparable, provides assurance of technical accuracy; or, if they differ, of the necessity for confirmation. The small percentage of sera which react with one procedure and not with another, when the methods approximate each other in sensitivity, certainly deserves further study.

Arrangements for sufficient standardization of complement-fixation tests and of precipitation tests also, at least to the extent that features generally recognized as desirable be incorporated in the procedures employed, would, I believe, tend markedly to improve the service in certain laboratories particularly some of those where the volume of the work done is not large. The establishment of means for providing reagents which could be used as standards should be especially helpful. Dr. Ruediger's suggestion relative to having available suitably preserved serums of designated titer, seems an important contribution.

It may not be amiss to reiterate the point made by Dr. Kilduffe that serological tests for syphilis should be conducted under the supervision of persons who are familiar with the symptomatology of the disease as well as with laboratory procedures. In the last analysis, the results of these, as of other diagnostic tests, must be interpreted in the light of clinical manifestations.

In view of what has already been accomplished, it is to be sincerely hoped

that adequate coöperation can be secured in perfecting and standardizing serological procedures so that every physician in this country who makes use of efficient laboratory service may feel assured that the most reliable data obtainable will be furnished him by his serologist.

B. S. KLINE. Personal experience with the Wassermann test by some half dozen technics in the past twenty-one years has revealed something of the temperamentality of the five ingredients, something of the mechanism of the reaction and something of the clinical application of the test. Seven years experience with various precipitation tests for syphilis has revealed something of the temperamentality of the two ingredients, something of the mechanism of the flocculation reaction and something of the clinical application of the tests.

The value of good Wassermann tests has been so amply demonstrated in the past twenty-five years that the state of flux mentioned by the Chairman has to do, I believe, with the value of the precipitation tests for syphilis. To what extent these tests will be developed and just what their ultimate clinical application will be is now a matter of opinion. At this time, I would like to discuss certain facts relating particularly to the precipitation tests.

In the first place, when some acetone insoluble fraction of alcoholic heart muscle extract either directly or from alcoholic solution is emulsified in a small quantity of water or salt solution, it is dispersed in the form of discrete globules averaging about 6 micra in diameter. Some of the globules are as large as 30 micra in diameter. If much water instead of a little is added, the dispersion of the antigen lipid is much greater and the particles are much smaller. If sufficient water is present, the dispersion is so great that the particles in great part are invisible through the microscope. The antigen in water, accordingly, behaves as a hydrophilic colloid.

The preparation of optimal antigen emulsion for tests for syphilis, the lipid is emulsified in a very small quantity of salt solution to insure the presence of the largest possible globules. When this emulsion is added to a strongly positive syphilitic serum, the latter causes a change in the surface of the globules and there results an aggregation of the altered globules. The globules may also be flocculated by alcohol (dehydration effect), by neutral salts, particularly bivalent and trivalent ones, and especially by acids (electrical neutralization or electrical neutralization and dehydration effects). As stated previously, it requires a strongly positive serum to flocculate the antigen globules. With weakly positive serum, flocculation does not occur and accordingly emulsions containing particles of antigen alone are insufficiently sensitive for serodiagnostic tests for syphilis.

In order to make the emulsions sufficiently sensitive, various substances are added to the antigen. In general, cholesterin is employed. Dr. Johns in his excellent slide test uses Balsam of Tolu as does Meinicke. Eagle in his test employs sitosterol as well as cholesterin. These adventitious substances upon

addition of water to their alcoholic solution, precipitate as clumps of crystals and behave characteristically as hydrophobic colloids. Although possessing no antigenic power, they greatly increase the sensitivity of antigen emulsions. As ordinarily employed, cholesterin or like substances is added to alcoholic solution of antigen and this combined solution is mixed with salt solution. As a result particles are formed composed of antigen and cholesterin, apparently uniformly distributed. It has been found that the size and shape of such particles depend especially upon the quantitative relation of the lipoids to water, upon the speed of mixture and upon the temperature of the ingredients. It is possible in chemically identical emulsions, for instance, to produce large needle like particles 60 micra or more in length by slowly mixing cold ingredients, and very small particles, 1 micron and less in diameter, by quickly mixing warm ingredients. Furthermore, because antigen tends to emulsify in water in discrete small globules and cholesterin or like substance tends to precipitate in clumps of crystals, particles produced as just stated are decidedly unstable and actually change in form and composition. In a few minutes to about eight hours at room temperature the changes are so marked that the emulsions are unsatisfactory for use. Within proper time limits, however, these flat particles of antigen and cholesterin or like substance, better adapted for agglutination than antigen globules alone, serve better than the latter in the serodiagnosis of syphilis.

When large needle-like particles are employed, as in the Müller-Ballung reaction, and in the "ball tests" demonstrated in the Scientific Exhibit, their marked alteration by strongly positive serum results in clumps with numerous needle-like projections which interlock upon sufficient agitation forming one large ball in clear fluid. If the particles are less altered, the ball formation is less pronounced and the fluid is somewhat turbid. With negative sera, the tests show a very turbid fluid and no clumps. The advantage of this type of reaction first reported by Hecht, in 1914, and employed by Müller in his Ballung test is that the result may be read accurately and easily with the naked eye. With the "ball tests," it is not necessary to call a conference to decide whether a test is negative or doubtful, doubtful or positive since the different reactions are strikingly distinctive.

Concerning the mechanism of the Kahn test, the Kahn antigen dilution is composed of clumps of numerous small particles averaging about 1 micron in diameter or less. In the presence of positive or negative serum, the fluid present leads to a breaking up of the clumps, the particles becoming discretely distributed in the fluid. If the serum is a positive one, the particles are immediately and intimately surrounded by reagin. The process is somewhat analogous to blotting a section and adding alcohol thereafter instead of adding alcohol to a water-soaked section. (The dehydration is much better than if the alcohol reached the tissue through a film of water.) If the serum is a negative one the particles remain discretely dispersed but if it is a positive one, the

preliminary discrete dispersion of the particles is followed after the surface alteration by a re-aggregation of the altered particles. (In his original test, Kahn employed completely dispersed particles in the antigen dilution. The test, however, required a long time for its completion.) Although the incomplete dispersion of antigen particles determines greater sensitivity by affording optimal conditions for alteration of particle surface by syphilitic serum, it seems more advisable to employ an emulsion of discrete flat particles of larger size requiring less reagin for formation of a visible aggregate. This latter principle is employed in most of the precipitation tests.

Inasmuch as the mixture of cholesterinized antigen solution and salt solution results in emulsions of particles varying greatly in size and shape depending upon the quantity of water, the temperature of ingredients and the speed of mixture particularly, emulsions so prepared are unavoidably variable since there is a definite relationship between the size, shape and number of antigen particles to the sensitivity of the emulsion. In contrast to such emulsions are those prepared by first precipitating the cholesterin in water and subsequently coating the cholesterin plates with antigen lipid. Because the quantity of water, the temperature of the ingredients and the speed of mixture exert no great influence upon cholesterin precipitation in water, the particles so prepared are more uniform in size and shape and when coated with antigen lipid determine emulsions giving more uniformly sensitive results in tests for syphilis. This principle is employed in the preparation of emulsions for the microscopic slide precipitation tests for syphilis. Furthermore, such emulsions are comparatively stable, being satisfactory for use for forty-eight hours.

The rôle of cholesterin in the antigen emulsion is very clearly shown by this method of preparation. Whereas with a fixed quantity of cholesterin in a given total the antigen may be varied as much as 400 per cent without appreciable change in results, the sensitivity of the emulsion is influenced greatly by the amount of cholesterin present. For instance, with 0.1 cc. of antigen in a total of 4.4 cc., a sensitive emulsion is determined by the addition of 0.75 cc. of 1 per cent cholesterin whereas with 1.25 cc. of 1 per cent cholesterin in the same formula a very sensitive emulsion is produced. By varying the quantity of cholesterin, then, the sensitivity of the emulsion may be set at any desired level. If too much cholesterin or other like adventitious substance is present, the emulsion may be too sensitive for reliable diagnostic purposes and the false positivity will be due not to the antigen but to the adventitious material.

Inasmuch as alcoholic tissue extracts contain abundant adventitious material as well as antigen lipid, it has been found necessary for reliable results to remove these substances as much as possible and to add a known quantity of cholesterin or like substance to obtain the desirable sensitivity. Dried tissues accordingly have been extracted with ether or acetone to remove the undesirable substances and the subsequent alcoholic extract has been employed as antigen. Kahn antigen is such a purified antigen. It has been found, however, that whereas

this antigen does not contain appreciable acetone soluble substances, it does contain ether insoluble adventitious substances, the quantity varying with different powders and different ethers employed. A method of more completely purifying antigen is that employed by Noguchi, Browning Cruikshank and McKenzie, Neymann and Gager, Kolmer and Kiss. This method consists in the addition of acetone to alcoholic tissue extract. The adventitious substances are soluble in the acetone and the small acetone insoluble fraction is the specific antigenic portion.

The antigen employed for the microscopic slide precipitation tests is such an acetone insoluble fraction of alcoholic heart powder extract and has been found not only potent and specific but likewise freer of adventitious material than antigen prepared by alcohol extraction of heart powder following preliminary ether or acetone extraction. It serves so well as a base for preparation of antigen emulsions of any desired sensitivity that it may well be worth while for the Society to determine definitely the value of this fraction of alcoholic tissue extract originally recommended by Noguchi.

In the preparation of such an antigen the conditions of the precipitation in acetone are of importance. After most of the alcohol of the tissue extract is evaporated it is poured into acetone. The purest fractions are obtained when the precipitation occurs in acetone at 50°C. to 37°C. for a period of fifteen minutes. Longer periods of precipitation and precipitation at lower temperatures permit of precipitation of adventitious substances as well and such antigens give more sensitive and less specific results.

Concerning the clinical application of serum tests for syphilis, as pointed out by Kolmer and borne out by our experience in early and in treated cases of syphilis, such tests are not sensitive enough rather than too sensitive.

In a study of 9000 tests, for instance, with 20 per cent of the sera from syphilitics, there were eighty-three false negative very sensitive slide tests in treated syphilis and three in primary syphilis and 248 false negative very sensitive Wassermann tests with the same antigen in treated syphilis and seven in primary syphilis. For this reason an even more sensitive slide test is now being employed more surely to exclude syphilis. The additional sensitivity is achieved by heating the antigen emulsion at 56°C., by centrifuging it and by using the larger particles only in proper quantity. With such an emulsion, reactions of some positivity are noted as early as the first week after the appearance of the primary lesion and in treated cases for as long as a year after tests of ordinary sensitivity give negative results.

With modern hospital practice necessitating frequent blood transfusions, the laboratory has the responsibility of preventing the transfer of syphilis by this means and just as it is deplorable that a single false positive blood test should occur to stigmatize an individual so would it be no less deplorable to give syphilis to a single individual by transfusion because of a false negative blood test.

HEMOGLOBIN STANDARDS*

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Estimations of hemoglobin often are unsatisfactory to the laboratory worker and the reports may be confusing to the clinician. Unfortunately, there is no unanimity of opinion concerning the technic of determination or manner of recording results. This confusion is due largely to the multiplicity of methods employed and to the fact that there is now on the market no hemoglobinometer reading in per cent having any scientific basis for the selection of the standard used. The following clinical methods for the estimation of hemoglobin are in use in this country: (1) oxygen combining power (Van Slyke); (2) iron content (Wong); (3) spectrophotometer (Sheard and Sanford); (4) comparison with colored standards: (a) tinted paper (Talquist, (b) glass replacing hemoglobin (Dare, Von Fleischl-Miescher), (c) carbon monoxide-hemoglobin (Haldane, Palmer), (d) acid hematin (original Sahli), (e) solutions to substitute for acid hematin, in the Sahli apparatus or in a Duboscq colorimeter (Felton's pyrogallate and Osgood and Haskins' iron-chromium solutions), and (f) glass to substitute for acid hematin (Bausch and Lomb-Newcomer, Klett, new Sahli, Haden-Hausser).

The oxygen capacity method employing the Van Slyke apparatus is accurate when carried out by an experienced and trained technician but is too complicated to be used as a routine clinical laboratory procedure. The determination of the quantity of iron by the new technic of Wong[18] is accurate, quite simple, and deserves much wider use. The spectrophotometer is expensive, must be calibrated with solutions of unstable crystallized hemoglobin and can be utilized only as a major laboratory procedure

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even when the human eye is replaced by a photoelectric cell as in the Sheard-Sanford[12] hemoglobinometer. Of the colorimetric methods, the Talquist is little better than guessing; the Dare is difficult to read accurately, and subject to wide errors; the Sahli with an acid hematin, yellow glass or correctly made artificial standard (Osgood-Haskins) is a good clinical method; the Duboscq colorimeter with a colored glass standard as in the Newcomer and Klett methods or with correct standard solutions is satisfactory for laboratory use; and the Haden-Hausser instrument has its place of usefulness.

TABLE 1

HEMOGLOBIN STANDARDS IN DIFFERENT HEMOGLOBINOMETERS READING IN PER CENT

INSTRUMENT	GRAMS EQUAL TO 100 PER CENT HEMOGLOBIN
Haldane	13.80
Oliver	15.00
Talquist	15.80
Dare (prior to 1926)	13.80
Dare (1926 to 1931)	16.90
Dare (1931 to —)	16.00
Bausch and Lomb-Newcomer (original)	16.90
Sahli (original)	17.20
Sahli (present)	14.00
Von Fleischl-Miescher	15.80
Klett	15.36

The estimation of oxygen capacity by means of the Van Slyke apparatus is the method of choice for research work and for determining and checking standards. The iron method is the only possible substitute; comparative estimations indicate that it gives results equally as accurate as the oxygen capacity method (table 1). Where an inexpensive laboratory instrument is required, the Sahli with a solid glass rod standard, or with the Osgood-Haskins[10] artificial standard is satisfactory, if calibrated in grams. The Haden-Hausser[4] hemoglobinometer is simple and accurate but unfortunately more expensive. For use only in the laboratory the Bausch and Lomb-Newcomer and Klett instru-

ments with glass or liquid standards are very good. The accuracy of all the colorimetric methods cited, however, is almost entirely dependent upon the calibration of the glass or solution used as a standard, and can be depended upon only when this has been done correctly.

With the oxygen capacity and the iron methods the hemoglobin readings are directly in grams. Until recently most hemoglobinometers have yielded readings in per cent with no two instruments in agreement as to what should be taken as equivalent to 100 per cent (table 2). In later models of the Von Fleischl-Miescher

TABLE 2

HEMOGLOBIN IN GRAMS PER 100 CC.

NUMBER	OXYGEN CAPACITY METHOD (VAN SLYKE)	IRON CONTENT METHOD (WONG)
	grams	*grams*
1	13.13	13.46
2	15.11	14.68
3	15.02	14.68
4	13.28	12.95
5	17.31	17.53
6	15.82	16.25
7	14.95	14.63
8	13.15	12.98
9	12.40	12.50
10	13.43	13.32
Average.....	14.36	14.30

and the new Bausch and Lomb-Newcomer apparatus, the amount of hemoglobin is determined in grams only, although figures given in the table are suggested for transposing the figures into per cent. The Klett and the new Sahli with solid glass rod standard give readings in grams as well as in per cent; the results with the Haden-Hausser instrument are calculated only in grams. The Von Fleischl-Miescher instrument is calibrated by comparison with a solution of unstable hemoglobin crystals; the new Bausch and Lomb-Newcomer by spectrophotometric determinations, and the Haden-Hausser by direct Van Slyke readings; the method of calibration of the Klett and Sahli instruments is not known to me.

It is apparent that with proper calibration any accurate colorimetric method can be transposed directly into grams. Thus Osgood and Haskins'[7,9] results in normal individuals reported in grams were obtained by calibrating their colorimetric procedure by direct comparison with readings on the Van Slyke instrument. Wintrobe's figures[16,17] were obtained with a Newcomer glass standard checked by the oxygen capacity method. It seems fair to require that all hemoglobinometers, reading either in per cent or in grams should have as their basis of calibration figures

TABLE 3

COMPARISON OF HEMOGLOBIN READINGS WITH OXYGEN CAPACITY AND IRON CONTENT METHODS AND HADEN-HAUSSER HEMOGLOBINOMETER

BLOOD NUMBER	OXYGEN CAPACITY METHOD (VAN SLYKE)	IRON METHOD (WONG)	HADEN-HAUSSER HEMOGLOBINOMETER
	*grams**	*grams**	*grams**
1	12.1	11.8	12.0
2	12.4	12.1	12.0
3	10.6	10.3	10.5
4	10.4	10.7	10.5
5	9.4	9.4	9.5
6	7.6	7.8	7.8
7	14.4	14.4	14.5
8	6.0	6.2	6.0
9	8.0	8.4	8.0
10	11.2	11.8	11.5
Average.....	10.23	10.29	10.23

* Grams per 100 cc.

obtained by direct Van Slyke readings or a determination of the iron content. Any hemoglobin determination would thus be designated or understood in the laboratory: hemoglobin = —— per cent (—— gms. by the direct Van Slyke or the iron content method); or hemoglobin = —— gms. (direct Van Slyke or iron content method) with the —— hemoglobinometer.

In table 3 is shown a series of determinations with the Haden-Hausser hemoglobinometer compared with both the oxygen capacity and iron methods. Such a comparison should be available for every hemoglobinometer used. So far as the technic of

hemoglobin estimations is concerned, one may say that several methods and instruments are satisfactory, but a method or instrument should only be employed which allows the translation of the readings into grams of hemoglobin as determined by the direct Van Slyke or the iron method. This point could easily be settled by common consent among laboratory workers.

The next problem concerns the amount of hemoglobin per hundred cubic centimeters of blood in normal individuals when determined by an accurate method. Haldane,[5] on the basis of a very small number of determinations found an average of 13.8 grams of hemoglobin in healthy normal men in England. He employed the oxygen capacity method in the Haldane blood-gas apparatus. This so-called Haldane standard (13.8 grams = 100 per cent) is frequently used in America, although every one has realized that normal men show a reading well over 100 per cent when this standard is used. Recently, Price-Jones[12] has found an average hemoglobin content of 112 per cent in normal men in Boston with this standard, using a colorimetric procedure calibrated by the gasometric method. The Haldane standard should never be used in this country, since with its use normal individuals will not have a color index of 1.00. The Williamson[14] standard should also be discarded since the average normal for men (16.92 grams) is much higher than other workers have found with other accurate methods available. The spectrophotometer used by Williamson was calibrated with hemoglobin crystals which are unstable and difficult to prepare in pure form. Hence the results obtained with it can not be compared with the most accurate clinical method (direct Van Slyke) now is common use. Williamson's table is of great value, however, in showing the relative hemoglobin content at different ages in the two sexes.

What is the hemoglobin content per hundred cubic centimeters of normal individuals in this country by the direct Van Slyke or iron content method? No large series of normals has been reported with the iron method. Using the oxygen capacity method I[3] found the average in forty normal men several years ago to be 15.4 grams. This series I have recently increased to seventy and for this number the average hemoglobin content is

15.34 grams per hundred cubic centimeters (table 4). Using the same method, Dill[2] found the average in forty men in Boston, 15.38 grams per hundred cubic centimeters and Sopkey[13] in India, 15.30 grams in a series of 121 men. These three series are the only ones reported in which all the determinations were done by the oxygen capacity method. Osgood and Haskins (Portland, Oregon) using their inorganic standard calibrated against direct Van Slyke readings reported an average hemoglobin value of 16 grams in 153 men.[6] Wintrobe and Muller (New Orleans) first reported an average of 15.85 in men using the Newcomer method,

TABLE 4

A Summary of Three Series of Simultaneous Estimations of Hemoglobin and Red Cells in Blood

Observer and date	Determinations		Mean red cell counts		Hemoglobin				
					Mean concentration		Coefficient		
	Men	Women	Men	Women	Men	Women	Men	Women	Men and women
							grams†	*grams*†	*grams*†
Osgood and Haskins* (1926–27)	153	100	5.60	4.80	16.0	13.69	14.29	14.29	14.29
Wintrobe* (1929–30)	100	50	5.85	4.93	17.0	13.76	14.53	13.97	14.25
Haden (1922 and 1932)	70	30	4.95	4.38	15.34	13.37	15.50	15.26	15.38

* Figures given in personal communication from authors.

† Grams per 100 cc.

calibrated by direct Van Slyke readings. Later Wintrobe[1,5] revised his figures to 17 grams in men since a mistake in calibration of his instrument had been found. The figures for women are, of course, lower. Figures reported for other parts of the world vary little from those cited.

The clinician is interested in the absolute values for hemoglobin in the blood, but is even more interested in the hemoglobin content relative to normal and in the amount relative to the number of red cells present. Such reports are of great value and are accurate, provided one can translate them at will into grams, which is always possible if an absolute figure is taken as 100 per

cent. The mean concentration of hemoglobin in normal men should be taken as 100 per cent. It is apparent that a different figure would have to be taken for women. In studying the various types of anemia, the clinician must know the relation of the amount of hemoglobin to the red cell count, namely, the color index, and in calculating the per cent of cells, 5,000,000 red cells are taken as 100 per cent of cells. It is thus necessary to take as 100 per cent for the hemoglobin the number of grams of hemoglobin found per hundred cubic centimeters of blood per five million cells (the hemoglobin coefficient). In determining a standard the erythrocyte count as well as the amount of hemoglobin must be known. Haldane and Williamson did not record the number of red blood cells. The Haldane and Williamson standards have already been discussed as impossible of acceptance on other grounds but this is an added reason why they should not be used. Unfortunately, also, no red cell counts are reported for the hemoglobin estimations of Dill. Only three large series of simultaneous estimations of hemoglobin and of red cells have been made in this country, those of Osgood and Haskins, (1926–1927), of Wintrobe (1929–1930) and of Haden (1922 and 1932). A summary of the findings in these three series is shown in table 4. Osgood and Haskins found the same hemoglobin coefficient in men and women (14.29 grams); Wintrobe found 14.53 grams in men and 13.97 in women; my results show 15.50 grams in men and 15.26 grams in women. The difference in hemoglobin per cell in men and women is small so it is unnecessary to use a different standard for the two sexes.

It is apparent from table 4 that the variation in the hemoglobin coefficient is due largely to differences in the erythrocyte counts. If all the figures are correct, apart from the variation in hemoglobin, there is evident a wide variation in red cell count in different parts of the country. This might well be, since with the higher red cell counts reported for the Scandinavian countries, the hemoglobin is much the same as in this county. I have found the number of red cells in normal men in Detroit (5,030,000), in Kansas City (4,880,000), and in Cleveland (5,030,000) approximately the same. Likewise I found that red cell counts done by medical

students in Kansas City on their own blood with Hausser-Levy counting chambers varied little from year to year as is indicated in table 5. Sixteen female students during this same period showed an average of 4,420,000, per cubic centimeters. Emerson[1] reported an average of 5,000,000 among 176 medical students in Baltimore. No large series have been reported from other centers.

The hemoglobin standard which I suggested in 1922 as 100 per cent was 15.6 grams (15.55 grams). I am now using 15.4 grams on the basis of the larger series (100 men and women) reported here, although there is actually a difference of only 0.12 gram per hundred cubic centimeters in the standard for the two groups.

TABLE 5

RED CELL COUNTS MADE BY MEDICAL STUDENTS IN KANSAS CITY

YEAR	NUMBER OF STUDENTS	AVERAGE COUNT
1924–1925	25	5.12
1925–1926	37	5.06
1926–1927	32	5.04
1927–1928	36	4.99
1928–1929	46	5.02
1929–1930	54	4.85
	Total: 230	Average: 4.997

My standard has been found to give normal color indices for other workers in Philadelphia, Boston, and Baltimore, and for me in Detroit, Kansas City and Cleveland.

How is one to explain the differences in the three reported series, each suggesting a different hemoglobin coefficient? This can not be done. Only further accurate studies in different parts of this country will determine to what extent the normal red cell count varies. Until some common figure is agreed upon, the selection of a single standard is impossible. Tables such as Osgood[8] has given for calculating percentages can not be universally used since they are correct only when the red cell counts and the hemoglobin are the same as he has found them in Portland.

It is necessary that some method be made available for reporting to clinicians the hemoglobin in per cent and for calculating accurately the color and saturation indices. To meet this need I have the following plan to offer. Only a method or instrument for the determination of hemoglobin should be used which reads in grams as calibrated by the direct Van Slyke or iron content method and all readings should be recorded in the laboratory in grams. In each laboratory, then, the mean value for hemoglobin and for the red cell count is determined for the place in which the laboratory is located. This is done easily and quite accurately by counting the red cells in ten or more normal adults and by determining simultaneously the hemoglobin in the same individuals with an instrument reading in grams per hundred cubic centimeters. The mean value for erythrocytes and for hemoglobin is thus found and the mean value for hemoglobin in grams per hundred cubic centimeters of blood per five million cells or per million cells is calculated.

The number of grams of hemoglobin so determined is taken as normal or 100 per cent for the laboratory in which the calibration is done. When so determined the hemoglobin percentage in a given blood will be the same everywhere and with every observer even though there may be a wide variation in absolute figures, and the color index in normal individuals always will be within normal limits (0.90 to 1.10). Using this plan, a hemoglobin report would read: hemoglobin = —— per cent (100 per cent = hemoglobin content of normal adult with 5,000,000 erythrocytes). If, on further investigation, it is found that the hemoglobin coefficient is actually everywhere the same, absolute figures can then be used. Until such a time, each worker may use a standard hemoglobin coefficient determined on the basis of hemoglobin estimations and red cell counts made in his own laboratory, and may report the amount of hemoglobin in per cent of this standard.

SUMMARY AND CONCLUSIONS

1. In making hemoglobin determinations only an instrument or method should be employed in which the results are recorded in

grams as determined by the oxygen capacity or iron content method.

2. For clinical purposes the reports of hemoglobin are best given in per cent of normal of a healthy adult with a red cell count of 5,000,000 cells.

3. The Haldane and Williamson standards can not be so transposed since in determining the standards no red cell counts were done.

4. There is a wide variation in hemoglobin and erythrocyte counts and consequently in the hemoglobin coefficient in the three large series (Haden, Osgood and Haskins, and Wintrobe) reported for normal men and women.

5. A satisfactory standard must give a color index within normal limits (0.90 to 1.10) in normal individuals.

6. In the present state of confusion, the hemoglobin coefficient should be determined for each laboratory and the hemoglobin should be reported only in per cent of this normal.

7. The hemoglobin percentage of a given blood when determined by this method is necessarily the same in all laboratories within the limits of technical error.

REFERENCES

(1) Emerson, C. P.: Clinical diagnosis, fourth ed. Philadelphia, J. P. Lippincott Co. 513 pp.

(2) Dill: Quoted by Price-Jones.

(3) Haden, R. L.: The normal hemoglobin standard. Jour. Am. Med. Assn., **79:** 1496–1497. 1922.

(4) Haden, R. L.: A new hemoglobinometer. Jour. Lab. & Clin. Med., **16:** 68–72. 1930.

(5) Haldane, John, and Smith, J. L.: The mass and oxygen capacity of the blood in man. Jour. Physiol., **25:** 331–343. 1899.

(6) Osgood, E. E.: Personal communication.

(7) Osgood, E. E.: Hemoglobin, color index, saturation index and volume index standards: redetermination based on the findings in 137 healthy young men. Arch. Int. Med., **37:** 685–706. 1926.

(8) Osgood, E. E.: Tables for calculation of color index, volume index and saturation index based on recently determined standards. Jour. Lab. & Clin. Med., **12:** 899–906. 1927.

(9) Osgood, E. E., and Haskins, D. D.: Relation between cell count, cell volume and hemoglobin content of venous blood of normal young women. Arch. Int. Med., **39:** 643–855. 1927.

(10) Osgood, E. E., Haskins, H. D., and Trotman, F. T.: A simplification of the Osgood-Haskins hemoglobin method. Jour. Lab. & Clin. Med., **16:** 482–486. 1931.

(11) Price-Jones, C.: The concentration of hemoglobin in normal human blood. Jour. Path. & Bact., **34:** 779–787. 1931.

(12) Sheard, C., and Sanford, A. H.: Photo-electrometer with one stage of amplification: as applied to the determination of hemoglobin. Jour. Am. Med. Assn., **93:** 1951–1957. 1929.

(13) Sopkey: Quoted by Price-Jones.

(14) Williamson, C. S.: Influence of age and sex on hemoglobin. Arch. Int. Med., **18:** 505–528. 1916.

(15) Wintrobe, M. M.: Hemoglobin standards in normal men. Proc. Soc. Exp. Biol. & Med., **26:** 848–851. 1929.

(16) Wintrobe, M. M.: Blood of normal young women residing in a subtropical climate. Arch. Int. Med., **45:** 287–301. 1930.

(17) Wintrobe, M. M., and Muller, M. W.: Normal blood determinations in the South. Arch. Int. Med., **43:** 96–113. 1929.

(18) Wong, S. Y.: Colorimetric determination of iron and hemoglobin in blood. II. Jour. Biol. Chem., **77:** 409–412. 1928.

EDITORIAL

The Friedman Hormone Test for Pregnancy

Although evidence of and tests for pregnancy have been studied for centuries, credit should be given to Aschheim and Zondek, who in 1928 proposed an accurate experimental method of determining this condition. Their method required that five or six immature, female, white mice, twenty-one days old, be given injections of varying quantities of urine of the woman in question. Injections were given subcutaneously on two successive days, three times a day. After 100 hours, the ovaries were examined for precocious corpora lutea and corpora hemorrhagica, their presence being an indication that the urine contained an excess of anterior pituitary hormone and therefore that the woman was pregnant.

The accuracy of this method has been confirmed repeatedly, and the test now rests among the most refined known in biology. Unfortunately the method is relatively tedious and requires access to a larger colony of mice than is generally available.

Friedman, a little more than a year after the important work of Aschheim and Zondek had been done, profiting by his knowledge of the peculiar nature of ovulation in the rabbit, devised a much simpler test for pregnancy. He injected 5 cc. of urine from pregnant woman into young, adult, female rabbits, and was able to demonstrate that this urine produced typical corpora hemorrhagica in the ovaries of the rabbits. Urine from nonpregnant women produced no such results.

Results of the Friedman test were first presented before a scientific assembly at the ninth annual convention of the American Society of Clinical Pathologists, by Reinhart and Scott. Their results were excellent. This presentation was followed by numerous reports which amply confirmed their observations. The society has therefore felt a peculiar interest in this test, and

TABLE 1

FRIEDMAN HORMONE PREGNANCY TESTS

TOTAL TESTS	POSITIVE	NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE	ECTOPIC—POSITIVE	ECTOPIC—NEGATIVE	CHORIOEPITHELIOMA OR HYDATID MOLE—POSITIVE	CHORIOEPITHELIOMA OR HYDATID MOLE—NEGATIVE	EARLIEST TEST. DAYS AFTER LAST MENSTRUATION	AMOUNT OF URINE (EACH INJECTION)	NUMBER OF INJECTIONS	DURATION OF TEST	ANIMALS DYING FROM INJECTIONS	CLINICAL PATHOLOGIST REPORTING
										cc.		*hours*		
27	18	9		2		1			35	10	1	36	1	M. D. Bell, Dallas, Texas
32	16	16	1[1]	1		1[2]	1		56	10	1	48	2	E. D. Funk & F. M. Light, Reading Hospital, Reading, Pa.
21	13	6	2		2				32	7–10	1	30		H. F. Hunt, Geisinger Memorial Hospital, Danville, Pa.
96	56	40			1				35	12	1	24[3] and 48	2	L. C. Todd, Charlotte, N. C.
16	7	9		1					22	7–20	1	48	2[4]	B. C. Shakford, Long Beach, California
40	18	22		1[5]					10	10	1	48		J. M. Moore, San Antonio, Texas
102[6]	55	47	3	2		2	1		10	7–12	1	30	2	B. C. Russum, Omaha, Nebraska
46	26	20	1[7]	1					4	10	1	48	4	J. C. Simpson, Morristown, Pa.
57	28	29		1					33	5–10	1	36	3	J. R. Van Atta, Albuquerque, New Mexico
10		10								7–10	1	30–40		T. Zbinden, Private Laboratory
34	30	3		1[8]					42	7	1	48		G. B. Kramer, Youngstown, Pa.
104	37	67		4[9]	1				14	7–10	1	30	3	J. E. Robinson, King's Daughters Hospital
74	31	43					1		12	12–20	1	48	1	M. B. Lyon, South Bend, Indiana

181	87	94	3	4	2	1			29	8–12	1	48	1	F. C. Payne, Dayton, Ohio
363	188	175		4	3	1	1		20 (after coitus)	10	2	39	6	G. D. Maner, Los Angeles, California
44	26	18			1		1		5	10	2	48	3	A. S. Giordano, South Bend, Indiana
95	56	39		1	1				21 (after coitus)	10	2	48	1	B. W. Rhamy, Fort Wayne Med. Laboratory
112	78	34		1	1	1			17	10	2	36–48	4	V. Cefaln, Seattle, Washington
14	5	9							42	16	1	30–36	2	C. R. Drake, Minneapolis, Minnesota
75	49	26			1		1			6	2	48		L. P. Hastings, Hartford, Connecticut
65	36	29			1				33	10–20	1	24–48	3	May Owen, Terrell Laboratories
135	54	78	2	1	2	4	1		8	10	2	48	2	W. Simpson, Dayton, Ohio
369	134	235	1	9						24	1	48		Gertrude Moore, Oakland, California
60	31	28		1		1		1[10]	33	8–10	4	36–48	3	A. G. Foord, Pasadena, California
109	45	60	1	1	1	1			30	10–20	1	48	4	B. S. Kline, Mt. Sinai Hospital, Cleveland, Ohio
24	15	9					1		28	10 and 5	2	36–48		E. M. Butt, Clinical Laboratory, Los Angeles, California

[1] Result uncertain at the time.
[2] Surgeon reported ovum had been dead for at least one month.
[3] Two rabbits for each test.
[4] One specimen from patient taking ergotin compound.
[5] Second specimen was positive.
[6] 59 were Aschheim-Zondek tests.
[7] Probably not false as abortion probably took place.
[8] Patient had dead foetus.
[9] Probably due to being unable to control collection of certain specimens.
[10] After operation, patient well after six months.

TABLE 1—*Concluded*

TOTAL TESTS	POSITIVE	NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE	ECTOPIC—POSITIVE	ECTOPIC—NEGATIVE	CHORIOEPITHELIOMA OR HYDATID MOLE—POSITIVE	CHORIOEPITHELIOMA OR HYDATID MOLE—NEGATIVE	EARLIEST TEST. DAYS AFTER LAST MENSTRUATION	AMOUNT OF URINE (EACH INJECTION)	NUMBER OF INJECTIONS	DURATION OF TEST	ANIMALS DYING FROM INJECTIONS	CLINICAL PATHOLOGIST REPORTING
										cc.		*hours*		
24	12	12								7	1	48		W. M. Sheppe, Wheeling Clinic, Wheeling, West Virginia
141[11]	66	75	2	4			1			4	6[12]	48	3	J. H. Littener, Nashville, Tennessee
8[13]	4	4							7	7		35		W. H. Bailey, Wesley Hospital, Oklahoma City, Oklahoma
204	92	112	3[14]	3	3				20 (after coitus)	5–20	1	24–48	6	T. B. Magath, Mayo Clinic, Rochester, Minnesota
865	423	442	5	15			3			10	1	48		H. L. Reinhart, Ohio State University, Columbus, Ohio
1,048[15]	790	258		48	68	12	6		14 (after coitus)	10	1	48	10	F. E. Sondern & I. J. Silverman, New York City
4,595	2,526	2,058	24	106	88	25	18	1					72	

[11] 61 were Aschheim-Zondek tests.

[12] Three times a day.

[13] Aschheim-Zondek tests also used.

[14] One false positive in patient with suprarenal tumor.

[15] Aschheim-Zondek tests on 487 simultaneously with Friedman as well as on many others in this series.

at the eleventh convention, authorized Dr. A. G. Foord, Chairman of the Committee on Research, to send out a questionnaire to all members of the Society requesting information as to their experience with this test. This information may be found in table 1, from which certain definite conclusions can be drawn. More extended consideration of the test will be found in two articles in this issue.

Although there have been many slight modifications of the procedure advocated by Friedman, it becomes evident that the test is satisfactorily performed when about 10 cc. of freshly passed, morning urine is injected into the marginal ear vein of a young, nonpregnant adult, female rabbit which has been isolated from male rabbits for at least three weeks. The observation of the ovaries of the rabbit is best made after forty-eight hours has elapsed, although after twenty-four hours the reaction is usually sufficient to make an observation trustworthy. To allow observation of the ovaries, the animal may be killed, or it may be operated on under anesthesia. If operation is performed the rabbit may be used again after one month.

Since information has been obtained on 4,595 tests, of which 55 per cent were positive and 45 per cent negative, one may reach very definite conclusions as to the value of the test. It is demonstrated, for instance, that 130 (2.83 per cent) results were erroneous. Unfortunately, as in the case of most questionnaires, the information given was not entirely accurate, since all observers probably did not report on exactly the same basis. There is, therefore, a very small percentage of error in these figures. Nevertheless, the largeness of the sample makes the reported results highly accurate. The tests which gave erroneous results are divided so that twenty-four (0.95 per cent) are false positives and 5.1 per cent are false negatives. Many perfectly logical explanations are offered for these errors. The most common false negative result is due to testing the urine before the woman has been pregnant long enough for the result to be positive in the rabbit. Other sources of error are failure to inject fresh urine, and failure to use sufficient quantities of urine. Some of the false positive results are due to failure to have the test rabbit

isolated from male rabbits, and perhaps occasionally to erroneous interpretation of the reaction. But any procedure which in a field test yields an uncorrected accuracy of more than 97 per cent, must be considered extremely satisfactory.

It was hoped that this test would yield accurate results in regard to ectopic pregnancy, in which condition accurate information is extremely important. Realizing the difficulties of verifying all such tests from a clinical and surgical standpoint, it is gratifying to note that the test has yielded an accuracy of 77.8 per cent, which, although the degree of accuracy is not so great as in uterine pregnancy, nevertheless compares very favorably with clinical diagnoses.

In the presence of chorio-epithelioma and hydatid mole the test gives extremely satisfactory results. There were nineteen such cases, in eighteen of which the test was positive. In regard to unusual malignant conditions of the testes, supposed to take origin from ovarian rests, information is not available.

An important consideration in hormone tests for pregnancy is the earliness with which the test will give a positive result. From the information available it becomes evident that the urine might contain sufficient hormone to produce a positive reaction in as short a time as fourteen days after impregnation. It seems quite evident that the test will be positive after three weeks.

From available information one must assume that about 1.5 per cent of the animals used will die, resulting in the failure of the test.

The Committee on Research and the Editor take the greatest pleasure and gratification in reporting these results, and thank the members of the Society who coöperated in making the report possible. It is evident that as a single laboratory procedure, on the basis of this rather large number of tests made by many different observers, the Friedman hormone test for pregnancy must be considered an outstanding contribution to the practice of medicine.

NEWS AND NOTICES

Training Schools for Technicians

The information contained in the tables on pages 104–106 deals with hospital and post-graduate training schools for technicians which have been approved through registration by the committee on Registry. It is furnished by Dr. Kano Ikeda and will be of great interest to clinical pathologists and laboratory technicians. Other applications are pending now and will be acted upon at the next annual convention.

Correction of Errors

The Editor wishes to call attention to two unfortunate errors in the list of members as published in the November issue of the Journal. Dr. F. E. Sondern should have been indicated as a Counsellor and Dr. A. V. St. George, who is a Fellow of the Society, should not have been indicated as an Associate Member.

Committee Appointments

The following Committees have been appointed:

Committee on Scientific Exhibit Awards

Dr. W. S. Thomas, Chairman
Dr. E. L. Miloslavich
Dr. H. C. Sweany

Committee on Local Arrangements

Dr. N. Enzer, Chairman (Hotel Accommodations)
Dr. E. L. Tharinger (Publicity)
Dr. E. F. Barta (Commercial Exhibits)
Dr. M. Fernan-Nunez (Scientific Exhibits)

Necrology Committee

Dr. J. J. Moore, Chairman
Dr. Herman Spitz
Dr. F. C. Payne

Committee on Necropsies

Dr. F. E. Sondern, Chairman
Dr. P. Hillkowitz
Dr. I. Davidsohn

Program Committee

Dr. A. S. Giordano, Chairman
Dr. A. H. Sanford
Dr. F. W. Hartman

Publication Committee

Dr. J. A. Kolmer, Chairman
Dr. Kano Ikeda
Dr. W. C. MacCarty

Research Committee

Dr. A. G. Foord, Chairman
Dr. B. S. Kline (Serology)
Dr. R. R. Kracke (Hematology)
Dr. O. A. Brines (Pathologic Anatomy)
Dr. I. A. Nelson (Biochemistry)
Dr. Ruth Gilbert (Bacteriology)
Dr. B. F. Stout (Vaccines)

Round Table Committee

Dr. A. G. Foord, Chairman
Dr. L. W. Larson
Dr. W. M. Sheppe

DATA ON TRAINING SCHOOLS FOR TECHNICIANS APPROVED THROUGH REGISTRATION BY THE BOARD OF REGISTRY

SCHOOL	ADDRESS	DIRECTOR	ENTRANCE REQUIREMENTS	LENGTH OF COURSE	FEES
Ancker Hospital Laboratory	St. Paul, Minnesota	Dr. John F. Noble	2 years university work	1 year	None
Archbold Memorial Hospital Laboratory	Thomasville, Georgia	Dr. M. J. Erickson	1 year college work	1 year	None
*Atlantic City Hospital Laboratory	Atlantic City, N. J.	Dr. R. A. Kilduffe	High school diploma	12–16 mo.	None
Bender Hygienic Laboratory	Albany, New York	Dr. A. W. Wright	1–2 years college work	1 year	$100.00
Buffalo General Hospital Laboratry	Buffalo, New York	Dr. Byron D. Bowen	College work	1 year	None
Henry Ford Hospital	Detroit, Michigan	Dr. F. W. Hartman	Degree from app. college or university	18 mo.	None
Dr. W. G. Gamble's Training School	Bay City, Michigan	Dr. W. G. Gamble	2 years college	12–18 mo.	$200.00
Training School Drs. Hillkowitz and Freshman	Metrop. Bldg., Denver, Colorado	Dr. Philip Hillkowitz	1 year college work	1 year	$100.00
*Dept. of Laboratories, House of Good Samaritan	Watertown, N. Y.	Dr. H. N. Cooper	High school graduate	18 mo.	None
Clinical Laboratory of Jefferson Hospital	Philadelphia, Pa.	Dr. B. L. Crawford	1 year college work	12–18 mo.	None
Minneapolis General Hospital	Minneapolis, Minnesota	Dr. N. H. Lufkin	2 years college work	1 year	$50.00
Charles T. Miller Hospital	St. Paul, Minnesota	Dr. Kano Ikeda	1 year college work	16 mo.	$125.00
*Monmouth Memorial Hospital	Long Branch, N. J.	Dr. C. A. Pons	High school diploma	1 year	None
*Mount Sinai Hospital	Cleveland, Ohio	Dr. B. S. Kline	High school diploma	1 year	None
*Mount Sinai Hospital	Philadelphia, Pa.	Dr. D. R. Meranze	High school diploma	18 mo.	$125.00
Newark Beth Israel Hospital	Newark, N. J.	Dr. Asher Yaguda	1 year college	1 year	None
Saint Joseph Hospital	Lexington, Ky.	Dr. E. S. Maxwell	1 year college	1 year	$50.00
Stuart Circle Hospital Labs	Richmond, Va.	Dr. Regena C. Beck	2 years college	1 year	$10.00

Swedish Hospital School of Technicians	914 So. 8th St., Minneapolis, Minnesota	Dr. Chas. R. Drake	1 year college	1 year	$100.00
Uniontown Hospital	Uniontown, Pa.	Dr. H. A. Heise	1 year college	2 years	Students paid $25 per mo.
*Univ. of California Hospital	San Francisco, Calif.	Dr. I. C. Schumacher	High school diploma	16 mo.	None
*Graduate Hospital Univ. of Pennsylvania	Philadelphia, Pa.	Dr. John A. Kolmer	High school or college graduate	18 mo.	$180.00
Wm. Pepper Laboratory of Clinical Medicine	Philadelphia, Pa.	Dr. Herbert Fox	1 year college	1 year	$150.00
*Wm. Volker Laboratory Training School	Kansas City, Mo.	Dr. Fred. C. Narr	High school diploma	1 year	None
Wisconsin General Hospital	Madison, Wisconsin	Dr. W. D. Stovall	College graduate	1 year	$25.00
Presbyterian Hospital	Denver, Colorado	Dr. Paul C. Carson	2 years college	1 year	None
St. Mary's Hospital	Duluth, Minnesota	Dr. Geo. Berdez	2 years college	1 year	$50.00
St. John's Hospital	Springfield, Illinois	Dr. Walter G. Bain	**Graduate nurse	1 year	$50.00
Sacred Heart Hospital	Spokane, Washington	Dr. M. M. Patton	1 year college	1 year	None

* Entrance requirements to be advanced to 1 year college work in chemistry and biology.

** Graduation in nursing to be considered the equivalent of 1 year college in chemistry and biology.

POST-GRADUATE OR COLLEGE COURSE IN MEDICAL TECHNOLOGY REGISTERED WITH THE BOARD

SCHOOL	ADDRESS	DIRECTOR	ENTRANCE REQUIREMENTS	LENGTH OF COURSE	FEES
Graduate School, Emory University	Emory University, Ga.	Dr. Roy R. Kracke	A.B. or B.S.	12–15 mo.	$40.00 per major
Michigan State College of Agriculture & Appl. Science	East Lansing, Michigan	Dr. Ward Giltner	High school diploma	4 years B.Sc.	Reg. College Fees
N. Y. Post Grad. Med. School	303 E. 20th St., New York City	Dr. Ward MacNeal	College graduate, graduate nurse	6 mo. (Cert. to M.D.'s only)	$250.00
N. Y. Univ. Med. College	338 E. 26th St., New York City	Dr. Samuel A. Brown	B.A. or B.S.	90 hours	
North Carolina College for Women	Greensboro, N. C.	Lila Belle Love	College entrance	4 years	Reg. College Tuition

BOOK REVIEWS

Laboratoriumstechnik in der Medizin. By Numerous Authors, Edited by Kurt Holm. Pp. xii + 714, 1932, Hamburg, Paul Hartung, 36m.

This volume has been prepared by twenty-one different authors whose contributions have been edited and arranged by Holm. It presents the laboratory side of medicine in a manner which is rather novel. The twenty chapters deal with all the various phases of clinical laboratory work, but there are special chapters on physical, chemical, histological and anatomical considerations not usually found in such manuals.

The topics covered by the chapter headings cannot be considered with any degree of detail within the bulk of the volume. However, enough detail is given so that the laboratory technician, for whom the volume has been primarily designed, is enabled to obtain quite a comprehensive general knowledge of the various questions concerned. The technical methods described are presented in a clear concise fashion. In a number of particulars the technic of individual examinations vary from the commonly accepted routine as practiced in the United States.

The illustrations, which are numerous, are for the most part original. They are well done and clearly illustrate the points in question.

The volume is of value to the laboratory technician who, while being familiar with the mechanical routine of laboratory procedures, desires to know more of the theory upon which the procedures are predicated. For those whose education has been more extensive in the chosen fields, the volume is of value in presenting under one cover a mass of information usually found only after search in many scattered volumes. The book, however, does not present the detailed description of methods and technic which would render it of value to advanced workers as an

additional reference book, but this was not the intention of the authors.

—G. L. ROHDENBURG

A Text-book of Pathology: An Introduction to Medicine. By WILLIAM BOYD. Pp. 946, 1932, Philadelphia, Lea & Febiger, $10.00.

The wide experience of the author as a teacher, has made him peculiarly fitted to write a text-book for students on the subject of Pathology. This text has been written from the standpoint of the student and is not intended to be an exhaustive reference book on the subject but the book is also intended for use by the clinician who wishes to brush up his knowledge of morbid anatomy. The text differs from the usual ones in pathology in that it presents the subject from the standpoint of physiology rather than from the standpoint of histology although this latter phase is by no means neglected. There is a definite attempt on the part of the author to coördinate his subject with general medicine and surgery and to that end he has included a description of the symptoms of diseases showing how they are related to the pathological processes. There has been a definite attempt to develop in the student who uses this text an attitude of mind which will cause him to seek correlation between pathology and symptoms.

The book is divided into general pathology and special pathology and instead of giving references, the author has given lists of related research for additional reading. The book is well written and in an extremely readable style. The illustrations, more than 275, are original and are excellently selected and reproduced. An unusually complete index makes the subject material immediately available to the casual reader.

Oral Spirochetes and Related Organisms in Fuso-Spirochetal Diseases. By DAVID T. SMITH. Pp. ix + 243, 1932, Baltimore, The Williams & Wilkins Company, $4.50.

This is the first comprehensive work on the subject and the first available in the English language. The author takes the position that "While there is no evidence that oral spirochetes

alone can produce disease in man, they are probably the most important member in a symbiotic group of anaërobic organisms capable of initiating a severe and often fatal disease."

After learning from the introduction that the clinical lesions are rather characteristic, the diagnosis easily made and the treatment fundamentally the same, one wonders why a monograph of this length should be written, but the necessity of such a book is apparent by casually scanning the 822 references at the end of the book and by carefully reading the text. The author has given an excellent description of the organisms involved, their method of growth and demonstration, the pathology they produce both naturally and experimentally and the method of prevention and treatment. One learns that these organisms cause disease in almost every part of the body and that these lesions are by no means infrequently encountered in laboratory and clinical practice. To the general clinician to pathologists and the oral specialist the book will prove of tremendous value. Although it is evident that the subject is by mo means closed, the author has revealed the state of knowledge up to the present time.

The Clay-Adams Company has specialized in hemacytometers and pipettes for many years and have advertised them in the JOURNAL since the first issue. These instruments are high class products and guaranteed to be accurate.